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(54) Title: REAGENTS AND METHODS FOR IDENTIFYING GENE TARGETS FOR TREATING CANCER

(57) Abstract: The invention provides methods and reagents for identifying mammalian genes necessary for tumor cell growth as targets for developing drugs that inhibit expression of said genes and inhibit tumor cell growth thereby.

REAGENTS AND METHODS FOR IDENTIFYING GENE TARGETS FOR TREATING CANCER

BACKGROUND OF THE INVENTION

This application claims priority to U.S. Provisional Application Serial No.: 60/306,730, filed July 20, 2001.

This application was supported by a grant from the National Institutes of Health, No. R01 CA62099. The government may have certain rights in this invention.

1. Field Of The Invention

The invention is related to methods and reagents for inhibiting tumor cell growth. Specifically, the invention identifies genes necessary for tumor cell growth as targets for developing drugs to inhibit such genes and thereby inhibit tumor growth. The invention provides methods for screening compounds to identify inhibitors of said genes, and methods for using said inhibitors to inhibit tumor cell growth. The invention also provides peptides encoded by genetic suppressor elements of the invention and mimetics and analogues thereof for inhibiting tumor cell growth. Also provided by the invention are normalized random fragment cDNA libraries prepared from tumor cells of one or a plurality of tumor cell types wherein the cDNA fragments can be induced by treating recipient cells with a physiologically-neutral stimulating agent.

2. Summary Of The Related Art

The completion of the draft sequence of the human genome has provided the art with a partial list of known and putative human genes, the total number of which is estimated to be between 30,000 and 45,000 (Venter *et al.*, 2001, *Science* 291: 1304-1351; Lander *et al.*, 2001, *Nature* 409: 860-921). These genes provide many potential targets for drugs, some of which may be useful in preventing the growth of cancers. However, the development of clinically useful gene-targeting anticancer drugs could be greatly facilitated by the ability to narrow down the list of human genes to those that are involved in the primary feature of cancer, uncontrolled tumor growth. It would be especially useful to identify genes necessary for the growth of tumor cells and to determine which of the genes play a

tumor-specific role and are not required for normal cell growth. These genes are particularly attractive targets for developing tumor-specific anticancer agents.

Most of the effort in tumor-specific drug targeting in the prior art has focused on oncogenes, the function of which has been associated with different forms of cancer Perkins and Stern (1997, *in* CANCER: PRINCIPLES AND PRACTICE OF ONCOLOGY, DeVita *et al.*, eds., (Philadelphia: Lippincott-Raven), pp. 79-102). Oncogene targets have been viewed in the art as being more "tumor-specific" than "normal" cellular enzymes that are targeted by the drugs used in present chemotherapeutic regimens. The tumor specificity of oncogenes has been suggested primarily by the existence of oncogene-associated genetic changes, such as mutations or rearrangements, specific to neoplastic cells. Although oncogenes are mutated or rearranged in some cases, in other cases they are merely expressed at elevated levels or at inappropriate stages of the cell cycle, without changes in the structure of the gene product (Perkins and Stern, 1997, *Id.*). Even when mutated, proteins encoded by oncogenes rarely acquire a qualitatively novel function relative to the "normal" protooncogene products. Hence, products of mutated, rearranged or overexpressed oncogenes generally perform the same biochemical functions as their normal cell counterparts, except that the functions of the activated oncogene products are abnormally regulated.

It is noteworthy that none of the "classical" oncogenes known in the art have been identified as targets for clinically useful anticancer drugs discovered by traditional mechanism-independent screening procedures. Rather the known cellular targets of chemotherapeutic drugs, such as dihydrofolate reductase (inhibited by methotrexate and other antifolates), topoisomerase II ("poisoned" by epipodophyllotoxins, anthracyclines or acridine drugs), or microtubules that form the mitotic spindle (the targets of *Vinca* alkaloids and taxanes) are essential for growth and proliferation of both normal and neoplastic cells. Tumor selectivity of anticancer drugs appears to be based not merely on the fact that their targets function primarily in proliferating cells, but rather on tumor-specific response to the inhibition of anticancer drug targets. For example, Scolnick and Halazonetis (2000, *Nature* 406 430-435) disclosed that a high fraction of tumor cell lines are deficient in a gene termed CHFR. In the presence of antimicrotubular drugs, CHFR appears to arrest the cell cycle in prophase. CHFR-deficient tumor cells, however, proceed into drug-impacted abnormal metaphase (Scolnick and

Halazonetis, 2000, *Id.*), where they die through mitotic catastrophe or apoptosis (Torres and Horwitz, 1998, *Cancer Res.* 58: 3620-3626). In addition to CHFR, tumor cells are frequently deficient in various cell cycle checkpoint controls, and exploiting these deficiencies is a major direction in experimental therapeutics (O'Connor, 1997, *Cancer Surv.* 29: 151-182; Pihan and Doxsey, 1999, *Semin. Cancer Biol.* 9: 289-302). In most cases, however, the reasons that inhibition of anticancer drug targets selectively induces cell death or permanent growth arrest in tumor cells are unknown. There is therefore need in the art to identify additional molecular targets in tumor cells, inhibition of which would arrest tumor cell growth.

One method known in the art for identifying unknown genes or unknown functions of known genes is genetic suppressor element technology, developed by some of the present inventors (*in* U.S. Patent Nos. 5,217,889, 5,665,550, 5,753,432, 5,811,234, 5,866,328, 5,942,389, 6,043,340, 6,060,134, 6,083,745, 6,083,746, 6,197,521, 6,268,134, 6,281,011 and 6,326,488, each of which is incorporated by reference in its entirety). Genetic suppressor elements (GSEs) are biologically active cDNA fragments that interfere with the function of the gene from which they are derived. GSEs may encode antisense RNA molecules that inhibit gene expression or peptides corresponding to functional protein domains, which interfere with protein function as dominant inhibitors. The general strategy for the isolation of biologically active GSEs involves the preparation of an expression library containing randomly fragmented DNA of the target gene or genes. This library is then introduced into recipient cells, followed by selection for the desired phenotype and recovery of biologically active GSEs from the selected cells. By using a single cDNA as the starting material for GSE selection, one can generate specific inhibitors of the target gene and map functional domains in the target protein. By using a mixture of multiple genes or the entire genome as the starting material, GSE selection allows one to identify genes responsible for a specific cellular function, since such genes will give rise to GSEs inhibiting this function. In a variation of this approach, the vector used for library preparation contains sequences permitting regulated expression of cDNA fragments cloned therein.

This method can be used to identify genes required for tumor cell growth by subjecting the cells to negative growth selection. One example of this type of

selection is known in the art as bromodeoxyuridine (BrdU) suicide selection, which has long been used to select conditional-lethal mutants (Stetten *et al.*, 1977, *Exp. Cell Res.* 108: 447-452) and growth-inhibitory DNA sequences (Padmanabhan *et al.*, 1987, *Mol. Cell Biol.* 7: 1894-1899). The basis of BrdU suicide selection is the destruction of cells that replicate their DNA in the presence of BrdU. BrdU is a photoactive nucleotide that incorporates into DNA and causes lethal DNA crosslinking upon illumination with white light in the presence of Hoechst 33342. The only cells that survive this selection are cells that do not replicate their DNA while BrdU is present, such as cells that express growth-inhibitory genes or GSEs. One advantage of this method is very low background of surviving cells. When used with GSE libraries under the control of an inducible vector, this selection method excludes spontaneously arising BrdU-resistant mutants by the insensitivity of their phenotype to the presence or absence of the inducing agent. Another major advantage of this technique is its sensitivity for weak growth-inhibitory GSEs: even if only a small fraction of GSE-containing cells are growth-inhibited by GSE induction, such cells will survive BrdU suicide and will give rise to a recovering clone.

The applicability of this approach to the isolation of growth-inhibitory GSEs was first demonstrated by Pestov and Lau (1994, *Proc. Natl. Acad. Sci. USA* 91: 12549-12553). These workers used an IPTG-inducible plasmid expression vector to isolate cytostatic GSEs from a mixture of cDNA fragments from 19 murine genes associated with the G_0/G_1 transition. In this work, three of the genes in the mixture gave rise to growth-inhibitory GSEs (Pestov and Lau, 1994, *Id.*). In a subsequent study, Pestov *et al.* (1998, *Oncogene* 17: 13187-3197) used the same approach to isolate one full-length and one truncated cDNA clone with growth-inhibitory activity from a 40,000-clone library of nominally full-length mouse cDNA. However, the method disclosed in the art cannot be efficiently used for transducing a library of random fragments representing the total mRNA population from a mammalian cell such as a tumor cell because the method relies on plasmid expression vectors for library construction, and only a limited number of cells can be stably transfected by such libraries.

There remains a need in the art to discover novel genes and novel functions of known genes necessary for tumor cell growth, especially by using methods for identifying genes based on function. There is also a need in the art to identify

targets for therapeutic drug treatment, particularly targets for inhibiting tumor cell growth, and to develop compounds that inhibit the identified targets and thereby inhibit tumor cell growth.

SUMMARY OF THE INVENTION

The present invention identifies genes that are targets for developing drugs for the treatment of cancer by inhibiting tumor cell growth. Such genes are identified as disclosed herein through expression selection of genetic suppressor elements (GSEs) that inhibit the growth of tumor cells *in vitro*. This selection has revealed multiple genes, some of which have been previously known to play a role in cell proliferation, whereas others were not known to be involved in cell proliferation prior to instant invention; the latter genes constitute novel drug targets and are set forth in Table 3.

In a first embodiment, the invention provides a method identifying a compound that inhibits growth of a mammalian cell, the method comprising the steps of:

(a) culturing a cell in the presence or absence of the compound;

(b) assaying the cell for expression or activity in the sample of one or a plurality of the genes set forth in Table 3; and

(c) identifying the compound when expression or activity in the sample of at least one of the genes set forth in Table 3 is lower in the presence of the compound than in the absence of the compound.

In preferred embodiments, the cell is a mammalian cell, preferably a human cell, and most preferably a human tumor cell. In further preferred embodiments, gene inhibition is detected by hybridization with a nucleic acid complementary to the gene, biochemical assay for an activity of the gene or immunological reaction with an antibody specific for an antigen comprising the gene product. In a preferred embodiment, the cell is a recombinant cell in which a reporter gene is operably linked to a promoter from a cellular gene in Table 3, to detect decreased expression of the reporter gene in the presence of the compound than in the absence of the compound. In further preferred embodiments, the cell is assayed for cell growth in the presence and absence of the compound, to identify compounds that inhibit cell growth and a gene identified in Table 3.

The invention also provides compounds that inhibit tumor cell growth that are identified by the methods of the invention, and pharmaceutical formulations of said compounds. The invention specifically provides peptides encoded by sense-oriented genetic suppressor elements of the invention. In addition the invention provides peptide mimetics comprising all or a portion of any of said peptides, peptido-, organo- or chemical mimetics thereof.

In a second embodiment, the invention provides a method for assessing efficacy of a treatment of a disease or condition relating to abnormal cell proliferation or tumor cell growth, comprising the steps of:

(a) obtaining a biological sample comprising cells from an animal having a disease or condition relating to abnormal cell proliferation or tumor cell growth before treatment and after treatment with a compound that inhibits expression or activity of a gene identified in Table 3;

(b) comparing expression or activity of at least one gene in Table 3 after treatment with the compound with expression or activity of said genes before treatment with the compound; and

(c) determining that said treatment with the compound has efficacy for treating the disease or condition relating to abnormal cell proliferation or neoplastic cell growth if expression or activity of at least one gene in Table 3 is lower after treatment than before treatment.

In preferred embodiments, the cell is a mammalian, most preferably human cell, most preferably a tumor cell.

In a third aspect, the invention provides a method for inhibiting tumor cell growth, the method comprising the steps of contacting a tumor cell with an effective amount of a compound that inhibits expression of a gene in Table 3.

In a fourth aspect, the invention provides a method for treating a disease or condition relating to abnormal cell proliferation or tumor cell growth, the method comprising the steps of administering to an animal having said disease or condition a therapeutically effective amount of a compound that inhibits expression of a gene in Table 3.

Pharmaceutically acceptable compositions effective according to the methods of the invention, comprising a therapeutically effective amount of a

peptide or peptide mimetic of the invention capable of inhibiting tumor cell growth and a pharmaceutically acceptable carrier or diluent, are also provided.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram illustrating the principles of genetic suppressor element technology.

Figure 2 is a schematic diagram of the structure of the LNCXCO3 retroviral vector.

Figure 3 is a schematic diagram of the BrdU selection protocol.

Figure 4 is a photograph of cell culture plates containing library-transduced cells subjected to BrdU suicide selection in the presence or in the absence of IPTG, immediately after G418 selection (top), after one round of BrdU suicide selection in the presence of IPTG (middle), or after two rounds of BrdU suicide selection in the presence of IPTG (bottom).

Figure 5 is a bar diagram of the results of testing of cell populations transduced with individual GSEs for IPTG-dependent resistance to BrdU suicide, measured in triplicates and expressed as mean and standard deviation of the numbers of colonies surviving BrdU suicide selection in the presence and in the absence of IPTG. Sequences for the shown results are GSE (SEQ ID NO):GBC-1 (79), GBC-3 (94), STAT3 (205), STAT5b (211), PRL31 (192), GBC-11 (85), L1CAM (125), INTB5 (112), OKCeta (170), VWF (225), ZIN (228), HSPCA (103), CDC20 (37), PKC zeta (172), CDK10 (39), DAP3 (59), RPA3 (190), NFkB1 (157), HES6 (99), and MBD1 (142).

Figure 6 is a bar diagram of the results of IPTG growth inhibition assays carried out with clonal cell lines transduced with individual GSEs, measured in triplicates and expressed as mean and standard deviation of the cell numbers after 7 days of culture in the presence and in the absence of IPTG. Sequences for the shown results are GSE (SEQ ID NO): HNRPF (101), HRMT1L2 (102), STAT5b (211), CCND1 (57), 28S RNA (17), RPL31 (192), CDK2 (40), AHRG (183), GBC-1 (79), L1CAM (125), NIN283 (158), MYL6 (155), DAP3 (59), TAF7 (215), STAT3 (205), IF1 (32), GBC-11 (85), LYN (138), c-KIT (48), GBC-3 (94), eIF-3

(62), PKCeta (170), EFNA1 (67), ATF4 (27), HNRPA2B1(102), GBC-12(86), INTB5 (112), BAM22 (35), FOS (43), FGFR1 (77), and KIAA1270 (123).

Figures 7A and 7B are photomicrographs illustrating the morphological effects of an L1CAM-derived GSE (SEQ ID NO 134) in a clonal IPTG-inhibited cell line. Figure 7A shows the effects on cell morphology of four-day treatment with IPTG. Figure 7B shows evidence of mitotic catastrophe in IPTG-treated cells.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

This invention provides target genes involved in cell growth, preferably tumor cell growth, methods for identifying compounds that inhibit expression or activity of these genes and methods for specifically inhibiting tumor cell growth by inhibiting expression or activity of these genes. Preferably, the methods of the invention do not substantially affect normal cell growth.

This invention provides methods for identifying genes that are required for tumor cell growth. Such genes, which are potential targets for new anticancer drugs, are identified through expression selection of genetic suppressor elements (GSEs). GSEs are biologically active sense- or antisense-oriented cDNA fragments that inhibit the function of the gene from which they are derived. Expression of GSEs derived from genes involved in cell proliferation is expected to inhibit cell growth. According to the inventive methods, such GSEs are isolated by so-called "suicide selection" of cells the growth of which is inhibited under cell culture conditions in which growing cells are specifically killed. In a preferred embodiment the suicide selection protocol is bromodeoxyuridine (BrdU) suicide selection, in which cells are incubated with BrdU and then illuminated with bright light. Growing cells incorporate BrdU into chromosomal DNA, making the DNA sensitive to illumination with light, which specifically kills growing cells. GSEs are produced starting from a normalized (reduced-redundance) library of human cDNA fragments in an inducible retroviral vector. In preferred embodiments, the recipient cells are tumor cells, most preferably human tumor cells, for example breast carcinoma cells.

For the purposes of this invention, reference to "a cell" or "cells" is intended to be equivalent, and particularly encompasses *in vitro* cultures of mammalian cells grown and maintained as known in the art.

For the purposes of this invention, reference to "cellular genes" in the plural is intended to encompass a single gene as well as two or more genes. It will also be understood by those with skill in the art that effects of modulation of cellular gene expression, or reporter constructs under the transcriptional control of promoters derived from cellular genes, can be detected in a first gene and then the effect replicated by testing a second or any number of additional genes or reporter gene constructs. Alternatively, expression of two or more genes or reporter gene constructs can be assayed simultaneously within the scope of this invention.

Recombinant expression constructs can be introduced into appropriate mammalian cells as understood by those with skill in the art. Preferred embodiments of said constructs are produced in transmissible vectors, more preferably viral vectors and most preferably retrovirus vectors, adenovirus vectors, adeno-associated virus vectors, and vaccinia virus vectors, as known in the art. See, generally, MAMMALIAN CELL BIOTECHNOLOGY: A PRACTICAL APPROACH, (Butler, ed.), Oxford University Press: New York, 1991, pp. 57-84.

In additionally preferred embodiments, the recombinant cells of the invention contain a construct encoding an inducible retroviral vector comprising random cDNA fragments from total tumor cell mRNA, wherein the fragments are each under the transcriptional control of an inducible promoter. In more preferred embodiments, the inducible promoter is responsive to a *trans*-acting factor whose effects can be modulated by an inducing agent. The inducing agent can be any factor that can be manipulated experimentally, including temperature and most preferably the presence or absence of an inducing agent. Preferably, the inducing agent is a chemical compound, most preferably a physiologically-neutral compound that is specific for the *trans*-acting factor. In the use of constructs comprising inducible promoters as disclosed herein, expression of the random cDNA fragments from the recombinant expression construct is mediated by contacting the recombinant cell with an inducing agent that induces transcription from the inducible promoter or by removing an agent that inhibits transcription from such promoter. A variety of inducible promoters and cognate *trans*-acting factors are known in the prior art, including heat shock promoters than can be activated by increasing the temperature of the cell culture, and more preferably promoter/factor pairs such as the *tet* promoter and fusions thereof with mammalian transcription factors (as are disclosed in U.S. Patent Nos. 5,654,168, 5,851,796,

and 5,968,773), and the bacterial *lac* promoter of the lactose operon and its cognate *lac*I repressor protein. In a preferred embodiment, the recombinant cell expresses the *lac*I repressor protein and a recombinant expression construct encoding the random cDNA fragments under the control of a promoter comprising one or a multiplicity of *lac*-responsive elements, wherein expression of the fragments can be induced by contacting the cells with the physiologically-neutral inducing agent, isopropylthio-β-galactoside. In this preferred embodiment, the *lac*I repressor is encoded by a recombinant expression construct identified as 3'SS (commercially available from Stratagene, LaJolla, CA).

The invention also provides recipient cell lines suitable for selection of growth-inhibitory GSEs. In preferred embodiments, the cell lines are human breast, lung, colon and prostate carcinoma cells, modified to comprise a *trans*-acting factor such as the *lac* repressor and further to express a retroviral receptor cognate to the tropism of the retroviral vector in which the library is constructed. In a preferred embodiment, the cells are modified to express the bacterial *lac* operon repressor, *lac*I (to allow for IPTG-inducible gene expression) and to express the ecotropic mouse retroviral receptor (to enable high-efficiency infection with ecotropic recombinant retroviruses). In alternative preferred embodiments, the cells are telomerase-immortalized normal human fibroblasts and retinal pigment and mammary epithelial cells that have been modified to express *lac*I and the mouse ecotropic retrovirus receptor.

The invention utilizes modifications of methods of producing genetic suppressor elements (GSEs) for identifying tumor cell growth controlling genes. These DNA fragments are termed "GSE" herein to designate both sense- and antisense-oriented gene fragments that can inhibit or modify the function of the target gene when expressed in a cell. Both types of functional GSEs can be generated by random fragmentation of the DNA of the target gene and identified by function-based selection of fragments that confer the desired cellular phenotype such as cell growth inhibition. Such function-based GSE selection makes it possible to develop genetic inhibitors for the selected targets, identify protein functional domains, and identify genes involved in various complex phenotypes.

A generalized scheme of GSE selection is shown in Figure 1. Originally developed using a model bacterial system (*see* U.S. Patent No. 5,217,889, incorporated by reference), this method has been adapted for use in mammalian

cells. Because less than 1% of random fragments derived from a typical cDNA have GSE activity, the size of expression libraries required for GSE selection is much larger than the corresponding size of libraries that can be used for function-based selection of full-length cDNAs. Retroviral vectors are used to deliver such large libraries into mammalian cells, because it is a non-stressful delivery system that can be used for stable transduction into a very high fraction (up to 100%) of recipient cells. In the preparation of these retroviral-based libraries, packaging cell lines are used, most preferably human 293-based packaging cell lines, such as BOSC23 (Pear *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90: 8392-8396), which provide efficient and uniform retrovirus packaging after transient transfection (Gudkov and Roninson, 1997, *in* METHODS IN MOLECULAR BIOLOGY: cDNA LIBRARY PROTOCOLS, Cowell and Austin, eds. (Totowa, N.J.: Humana Press), pp. 221-240). Additionally, large-scale expression selection required modifications in conventional retroviral vectors. The retroviral vectors used to produce the normalized tumor libraries of the invention carry one constitutively expressing and one inducible promoter, which minimizes the problem of promoter interference under non-inducing conditions. Preferred embodiments of the modified retroviral vectors of the invention express the bacterial neomycin resistance gene (*neo*, selectable in mammalian cells with G418) from an LTR promoter in the retrovirus. The vectors also contain a multiple cloning site 3' to the selectable marker gene and adjacent to a regulatable promoter comprising promoters from cytomegalovirus (CMV) or Rous sarcoma virus (RSV) LTR containing 2-4 bacterial *lac* operator sequences. The regulatable promoter is cloned in the *anti* orientation to the retroviral LTR. A diagram of the topography of one of these viruses, LNXCO3 is shown in Figure 2. In alternative embodiments, the *neo* gene is exchanged for a gene encoding green fluorescent protein (Kandel *et al.*, 1997, *Somat. Cell Genet.* 23: 325-340) or firefly luciferase (Chang *et al.*, 1999, *Oncogene* 18: 4808-4818). As a positive control for growth inhibition an embodiment of LNXCO3 was used that expressed human p21, a CDK inhibitor know to strongly inhibit tumor cell growth (*see* International Patent Application, Publication No. WO01/38532, incorporated by reference).

The invention provides a normalized cDNA fragment library from a mixture of poly(A)+ RNA preparations from one or a multiplicity of human cell lines, derived from different types of cancer. This normalized library is prepared in

a vector, preferably a retroviral vector and most preferably a retroviral vector containing sequences permitting regulated expression of cDNA fragments cloned therein. In a preferred embodiment, the vector is the retroviral vector LNXCO3, comprising a promoter inducible by isopropyl-β-thio-galactoside (IPTG), a physiologically neutral agent.

The invention provides methods for isolating growth-inhibitory GSEs from a normalized cDNA fragment library, representing most of the expressed genes in a human tumor cell. As provided herewith, normalized cDNA fragment libraries contain on the order of 5 x 10^7 clones (Gudkov *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91: 3644-3748; Levenson *et al.*, 1999, *Somat. Cell Molec. Genet.* 25: 9-26), corresponding to >1,000 cDNA fragments per gene. Selection of individual GSEs from a library of this size requires a procedure with high sensitivity and low background, most preferably BrdU suicide selection. The principle of BrdU suicide selection is illustrated in Figure 3. In preferred embodiments, the GSEs are expressed under the control of an inducible promoter, most preferably a promoter that is inducible by a physiologically neutral agent (such as IPTG), provided that the growth inhibitor is induced prior to the addition of BrdU. Following BrdU selection, the inducer is washed from the culture and cells infected with growth-inhibitory GSEs begin to proliferate, thus providing colonies of cells harboring selected GSEs.

BrdU suicide is not the only technique that can be used to select growth-inhibitory genes or GSEs. In one alternative approach, cells are labeled with a fluorescent dye that integrates into the cell membrane and is redistributed between daughter cells with each round of cell division. As a result, cells that have divided the smallest number of times after labeling show the highest fluorescence and can be isolated by FACS (Maines *et al.*, 1995, *Cell Growth Differ.* 6: 665-671). It is also possible to isolate cells that die upon the addition of the inducer, by collecting floating dead cells or isolating apoptotic cells on the basis of altered staining with DNA-binding fluorescent dyes. These methods have been used to isolate GSEs from single-gene cDNA fragment libraries prepared from the MDR1 gene (Zuhn, 1996, Ph.D. Thesis, Department of Genetics, University of Illinois at Chicago, Chicago, IL) or from BCL2 (U.S. Patent 5,789,389, incorporated by reference). There are no theoretical problems with any of these approaches, and all of them

work to enrich for growth-inhibitory elements in low-complexity libraries. The only disadvantage of these alternatives when compared with BrdU selection is that they have higher spontaneous background rates that may prevent rare clones to be selected from an exceedingly complex normalized library. Thus, BrdU selection is the preferred embodiment of the inventive methods.

Prior art methods (Pestov and Lau, 1994, *Id.*) for adapting GSE technology to identify growth-inhibitory GSEs were of limited utility when applied to total tumor cell cDNA. The prior art methods cannot be efficiently used for transducing a library representing the total mRNA population from a mammalian cell such as a tumor cell because the method relies on plasmid expression vectors for library construction, and only a limited number of cells can be stably transfected by such libraries. To overcome this limitation, the invention provides a set of inducible retroviral vectors that are regulated by IPTG through the bacterial *Lac*I repressor. This inducible system provides comparable levels of induction among most of the infected cells. The induced levels of expression can be finely regulated by using different doses of IPTG.

The methods of the invention are exemplified herein by use of this IPTG-inducible retroviral system to generate a normalized cDNA library from human breast cancer cells. This library was used to select GSEs that induce growth arrest in a breast carcinoma cell line. Using this approach, more than 90 genes were identified that were enriched by BrdU suicide selection. Many of these GSEs were shown to have a growth-inhibiting effect when re-introduced into tumor cells. Included in the genes identified using the inventive methods are known oncogenes, some of which have been specifically associated with breast cancer, as well as other genes with a known role in cell proliferation. Many of the identified genes, however, had no known function or were not previously known to play a role in cell cycle progression. The latter genes and their products represent therefore novel targets for cancer treatment. Furthermore, some of the genes giving rise to the GSEs that inhibited the proliferation of breast carcinoma cells appear to be inessential for normal cell growth, since homozygous knockout of these genes does not prevent the development of adult mice.

The invention provides methods for measuring gene expression or activity of the gene products corresponding to GSEs identified using GSE libraries and negative growth selection methods of the invention. In the practice of this aspect

of the methods of the invention, gene expression or gene product activity is assayed in cells in the presence or absence of a compound to determine whether the compound inhibits expression or activity of such a gene or gene product. In preferred embodiments, gene expression is assayed using any technique known in the art, such as comparison of northern blot hybridization to cellular mRNA using a detectably-labeled probe (as disclosed, for example, in Sambrook *et al.*, 2001, MOLECULAR CLONING: A LABORATORY MANUAL, 3rd ed., Cold Strong Harbor Laboratory Press: N.Y.), or by *in vitro* amplification methods, such as quantitative reverse transcription - polymerase chain reaction (RT-PCR) assays as disclosed by Noonan *et al.* (1990, *Proc. Natl. Acad. Sci. USA* 87: 7160-7164), or by western blotting using antibodies specific for the gene product (Sambrook *et al.*, 2001, *Id.*). Gene product activity is assayed using assays specific for each gene product, such as immunoassay using antibodies specific for said gene products or biochemical assay of gene product function.

Alternatively, gene expression is assayed using recombinant expression constructs having a promoter from a gene corresponding to GSEs identified using GSE libraries and negative growth selection methods of the invention, wherein the promoter is operably linked to a reporter gene. The reporter gene is then used as a sensitive and convenient indicator of the effects of test compounds on gene expression, and enables compounds that inhibit expression or activity of genes required for cell, preferably tumor cell growth to be easily identified. Host cells for these constructs include any cell expressing the corresponding growth-promoting gene. Reporter genes useful in the practice of this aspect of the invention include but are not limited to firefly luciferase, Renilla luciferase, chloramphenicol acetyltransferase, beta-galactosidase, green fluorescent protein, and alkaline phosphatase.

The invention provides peptides encoded by some of the GSEs of the invention that have been identified using the GSE-negative growth selection methods disclosed herein. Such peptides are presented in Table 5 and in the Sequence Listing as SEQ ID NOS. 229-314. Some of these peptides are derived from proteins that were previously known to play a role in cell proliferation, and others from proteins that were first assigned such a role in the instant inventions. All of the identified peptides, however, are novel inhibitors of tumor cell proliferation. Also provided are related compounds within the understanding of

those with skill in the art, such as chemical mimetics, organomimetics or peptidomimetics. As used herein, the terms "mimetic," "peptide mimetic," "peptidomimetic," "organomimetic" and "chemical mimetic" are intended to encompass peptide derivatives, peptide analogues and chemical compounds having an arrangement of atoms is a three-dimensional orientation that is equivalent to that of a peptide encoded by a GSE of the invention. It will be understood that the phrase "equivalent to" as used herein is intended to encompass compounds having substitution of certain atoms or chemical moieties in said peptide with moieties having bond lengths, bond angles and arrangements thereof in the mimetic compound that produce the same or sufficiently similar arrangement or orientation of said atoms and moieties to have the biological function of the peptide GSEs of the invention. In the peptide mimetics of the invention, the three-dimensional arrangement of the chemical constituents is structurally and/or functionally equivalent to the three-dimensional arrangement of the peptide backbone and component amino acid sidechains in the peptide, resulting in such peptido-, organo- and chemical mimetics of the peptides of the invention having substantial biological activity. These terms are used according to the understanding in the art, as illustrated *for example* by Fauchere, 1986, *Adv. Drug Res.* 15: 29; Veber & Freidinger, 1985, *TINS* p.392; and Evans *et al.*, 1987, *J. Med. Chem.* 30: 1229, incorporated herein by reference.

It is understood that a pharmacophore exists for the biological activity of each peptide GSE of the invention. A pharmacophore is understood in the art as comprising an idealized, three-dimensional definition of the structural requirements for biological activity. Peptido-, organo- and chemical mimetics can be designed to fit each pharmacophore with current computer modeling software (computer aided drug design). Said mimetics are produced by structure-function analysis, based on the positional information from the substituent atoms in the peptide GSEs of the invention.

Peptides as provided by the invention can be advantageously synthesized by any of the chemical synthesis techniques known in the art, particularly solid-phase synthesis techniques, for example, using commercially-available automated peptide synthesizers. The mimetics of the present invention can be synthesized by solid phase or solution phase methods conventionally used for the synthesis of peptides (*see, for example*, Merrifield, 1963, *J. Amer. Chem. Soc.* 85: 2149-54;

Carpino, 1973, *Acc. Chem. Res.* 6: 191-98; Birr, 1978, ASPECTS OF THE MERRIFIELD PEPTIDE SYNTHESIS, Springer-Verlag: Heidelberg; THE PEPTIDES: ANALYSIS, SYNTHESIS, BIOLOGY, Vols. 1, 2, 3, 5, (Gross & Meinhofer, eds.), Academic Press: New York, 1979; Stewart *et al.*, 1984, SOLID PHASE PEPTIDE SYNTHESIS, 2nd. ed., Pierce Chem. Co.: Rockford, Ill.; Kent, 1988, *Ann. Rev. Biochem.* 57: 957-89; and Gregg *et al.*, 1990, *Int. J. Peptide Protein Res.* 55: 161-214 , which are incorporated herein by reference in their entirety.)

The use of solid phase methodology is preferred. Briefly, an N-protected C-terminal amino acid residue is linked to an insoluble support such as divinylbenzene cross-linked polystyrene, polyacrylamide resin, Kieselguhr/polyamide (pepsyn K), controlled pore glass, cellulose, polypropylene membranes, acrylic acid-coated polyethylene rods or the like. Cycles of deprotection, neutralization and coupling of successive protected amino acid derivatives are used to link the amino acids from the C-terminus according to the amino acid sequence. For some synthetic peptides, an FMOC strategy using an acid-sensitive resin may be used. Preferred solid supports in this regard are divinylbenzene cross-linked polystyrene resins, which are commercially available in a variety of functionalized forms, including chloromethyl resin, hydroxymethyl resin, paraacetamidomethyl resin, benzhydrylamine (BHA) resin, 4-methylbenzhydrylamine (MBHA) resin, oxime resins, 4-alkoxybenzyl alcohol resin (Wang resin), 4-(2',4'-dimethoxyphenylaminomethyl)-phenoxymethyl resin, 2,4-dimethoxybenzhydryl-amine resin, and 4-(2',4'-dimethoxyphenyl-FMOC-amino-methyl)-phenoxyacetamidonorleucyl-MBHA resin (Rink amide MBHA resin). In addition, acid-sensitive resins also provide C-terminal acids, if desired. A particularly preferred protecting group for alpha amino acids is base-labile 9-fluorenylmethoxy-carbonyl (FMOC).

Suitable protecting groups for the side chain functionalities of amino acids chemically compatible with BOC (t-butyloxycarbonyl) and FMOC groups are well known in the art. When using FMOC chemistry, the following protected amino acid derivatives are preferred: FMOC-Cys(Trit), FMOC-Ser(But), FMOC-Asn(Trit), FMOC-Leu, FMOC-Thr(Trit), FMOC-Val, FMOC-Gly, FMOC-Lys(Boc), FMOC-Gln(Trit), FMOC-Glu(OBut), FMOC-His(Trit), FMOC-Tyr(But), FMOC-Arg(PMC (2,2,5,7,8-pentamethylchroman-6-sulfonyl)), FMOC-Arg$(BOC)_2$, FMOC-Pro, and FMOC-Trp(BOC). The amino acid residues can be

coupled by using a variety of coupling agents and chemistries known in the art, such as direct coupling with DIC (diisopropyl-carbodiimide), DCC (dicyclohexylcarbodiimide), BOP (benzotriazolyl-N-oxytrisdimethylaminophosphonium hexa-fluorophosphate), PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluoro-phosphate), PyBrOP (bromo-tris-pyrrolidinophosphonium hexafluorophosphate); via performed symmetrical anhydrides; via active esters such as pentafluorophenyl esters; or via performed HOBt (1-hydroxybenzotriazole) active esters or by using FMOC-amino acid fluoride and chlorides or by using FMOC-amino acid-N-carboxy anhydrides. Activation with HBTU (2-(1H-benzotriazole-1-yl),1,1,3,3-tetramethyluronium hexafluorophosphate) or HATU (2-(1H-7-aza-benzotriazole-1-yl),1,1,3,3-tetramethyluronium hexafluoro-phosphate) in the presence of HOBt or HOAt (7-azahydroxybenztriazole) is preferred.

The solid phase method can be carried out manually, although automated synthesis on a commercially available peptide synthesizer (*e.g.*, Applied Biosystems 431A or the like; Applied Biosystems, Foster City, CA) is preferred. In a typical synthesis, the first (C-terminal) amino acid is loaded on the chlorotrityl resin. Successive deprotection (with 20% piperidine/NMP (N-methylpyrrolidone)) and coupling cycles according to ABI FastMoc protocols (ABI user bulletins 32 and 33, Applied Biosystems are used to build the whole peptide sequence. Double and triple coupling, with capping by acetic anhydride, may also be used.

The synthetic mimetic peptide is cleaved from the resin and deprotected by treatment with TFA (trifluoroacetic acid) containing appropriate scavengers. Many such cleavage reagents, such as Reagent K (0.75 g crystalline phenol, 0.25 mL ethanedithiol, 0.5 mL thioanisole, 0.5 mL deionized water, 10 mL TFA) and others, can be used. The peptide is separated from the resin by filtration and isolated by ether precipitation. Further purification may be achieved by conventional methods, such as gel filtration and reverse phase HPLC (high performance liquid chromatography). Synthetic calcitonin mimetics according to the present invention may be in the form of pharmaceutically acceptable salts, especially base-addition salts including salts of organic bases and inorganic bases. The base-addition salts of the acidic amino acid residues are prepared by treatment of the peptide with the appropriate base or inorganic base, according to procedures

well known to those skilled in the art, or the desired salt may be obtained directly by lyophilization out of the appropriate base.

Generally, those skilled in the art will recognize that peptides as described herein may be modified by a variety of chemical techniques to produce compounds having essentially the same activity as the unmodified peptide, and optionally having other desirable properties. For example, carboxylic acid groups of the peptide may be provided in the form of a salt of a pharmaceutically-acceptable cation. Amino groups within the peptide may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be converted to an amide. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides of this invention so that the native binding configuration will be more nearly approximated. For example, a carboxyl terminal or amino terminal cysteine residue can be added to the peptide, so that when oxidized the peptide will contain a disulfide bond, thereby generating a cyclic peptide. Other peptide cyclizing methods include the formation of thioethers and carboxyl- and amino-terminal amides and esters.

Specifically, a variety of techniques are available for constructing peptide derivatives and analogues with the same or similar desired biological activity as the corresponding peptide compound but with more favorable activity than the peptide with respect to solubility, stability, and susceptibility to hydrolysis and proteolysis. Such derivatives and analogues include peptides modified at the N-terminal amino group, the C-terminal carboxyl group, and/or changing one or more of the amido linkages in the peptide to a non-amido linkage. It will be understood that two or more such modifications can be coupled in one peptide mimetic structure (*e.g.*, modification at the C-terminal carboxyl group and inclusion of a -CH_2- carbamate linkage between two amino acids in the peptide).

Amino terminus modifications include alkylating, acetylating, adding a carbobenzoyl group, and forming a succinimide group. Specifically, the N-terminal amino group can then be reacted to form an amide group of the formula RC(O)NH-- where R is alkyl, preferably lower alkyl, and is added by reaction with an acid halide, RC(O)Cl or acid anhydride. Typically, the reaction can be

conducted by contacting about equimolar or excess amounts (*e.g.*, about 5 equivalents) of an acid halide to the peptide in an inert diluent (*e.g.*, dichloromethane) preferably containing an excess (*e.g.*, about 10 equivalents) of a tertiary amine, such as diisopropylethylamine, to scavenge the acid generated during reaction. Reaction conditions are otherwise conventional (*e.g.*, room temperature for 30 minutes). Alkylation of the terminal amino to provide for a lower alkyl N-substitution followed by reaction with an acid halide as described above will provide for N-alkyl amide group of the formula RC(O)NR–. Alternatively, the amino terminus can be covalently linked to succinimide group by reaction with succinic anhydride. An approximately equimolar amount or an excess of succinic anhydride (*e.g.*, about 5 equivalents) are used and the terminal amino group is converted to the succinimide by methods well known in the art including the use of an excess (*e.g.*, ten equivalents) of a tertiary amine such as diisopropylethylamine in a suitable inert solvent (*e.g.*, dichloromethane), as described in Wollenberg *et al.*, U.S. Pat. No. 4,612,132, is incorporated herein by reference in its entirety. It will also be understood that the succinic group can be substituted with, for example, C_2- through C_6- alkyl or --SR substituents, which are prepared in a conventional manner to provide for substituted succinimide at the N-terminus of the peptide. Such alkyl substituents are prepared by reaction of a lower olefin (C_2- through C_6- alkyl) with maleic anhydride in the manner described by Wollenberg *et al.*, *supra.*, and --SR substituents are prepared by reaction of RSH with maleic anhydride where R is as defined above. In another advantageous embodiments, the amino terminus is derivatized to form a benzyloxycarbonyl-NH- - or a substituted benzyloxycarbonyl-NH-- group. This derivative is produced by reaction with approximately an equivalent amount or an excess of benzyloxycarbonyl chloride (CBZ-Cl) or a substituted CBZ-Cl in a suitable inert diluent (*e.g.*, dichloromethane) preferably containing a tertiary amine to scavenge the acid generated during the reaction. In yet another derivative, the N-terminus comprises a sulfonamide group by reaction with an equivalent amount or an excess (*e.g.*, 5 equivalents) of R--$S(O)_2Cl$ in a suitable inert diluent (dichloromethane) to convert the terminal amine into a sulfonamide, where R is alkyl and preferably lower alkyl. Preferably, the inert diluent contains excess tertiary amine (*e.g.*, ten equivalents) such as diisopropylethylamine, to scavenge the acid generated during reaction. Reaction conditions are otherwise conventional (*e.g.*, room temperature

for 30 minutes). Carbamate groups are produced at the amino terminus by reaction with an equivalent amount or an excess (*e.g.*, 5 equivalents) of R--OC(O)Cl or R--OC(O)OC_6H_4--*p*--NO_2 in a suitable inert diluent (*e.g.*, dichloromethane) to convert the terminal amine into a carbamate, where R is alkyl, preferably lower alkyl. Preferably, the inert diluent contains an excess (*e.g.*, about 10 equivalents) of a tertiary amine, such as diisopropylethylamine, to scavenge any acid generated during reaction. Reaction conditions are otherwise conventional (*e.g.*, room temperature for 30 minutes). Urea groups are formed at the amino terminus by reaction with an equivalent amount or an excess (*e.g.*, 5 equivalents) of R--N=C=O in a suitable inert diluent (*e.g.*, dichloromethane) to convert the terminal amine into a urea (*i.e.*, RNHC(O)NH--) group where R is as defined above. preferably, the inert diluent contains an excess (e.g., about 10 equivalents) of a tertiary amine, such as diisopropylethylamine. Reaction conditions are otherwise conventional (*e.g.*, room temperature for about 30 minutes).

In preparing peptide mimetics wherein the C-terminal carboxyl group is replaced by an ester (*e.g.*, --C(O)OR where R is alkyl and preferably lower alkyl), resins used to prepare the peptide acids are employed, and the side chain protected peptide is cleaved with base and the appropriate alcohol, *e.g.*, methanol. Side chain protecting groups are then removed in the usual fashion by treatment with hydrogen fluoride to obtain the desired ester. In preparing peptide mimetics wherein the C-terminal carboxyl group is replaced by the amide --C(O)NR_3R_4, a benzhydrylamine resin is used as the solid support for peptide synthesis. Upon completion of the synthesis, hydrogen fluoride treatment to release the peptide from the support results directly in the free peptide amide (*i.e.*, the C-terminus is --C(O)NH_2). Alternatively, use of the chloromethylated resin during peptide synthesis coupled with reaction with ammonia to cleave the side chain Protected peptide from the support yields the free peptide amide and reaction with an alkylamine or a dialkylamine yields a side chain protected alkylamide or dialkylamide (*i.e.*, the C-terminus is --C(O)NRR_1, where R and R_1 are alkyl and preferably lower alkyl). Side chain protection is then removed in the usual fashion by treatment with hydrogen fluoride to give the free amides, alkylamides, or dialkylamides.

In another alternative embodiment, the C-terminal carboxyl group or a C-terminal ester can be induced to cyclize by displacement of the --OH or the ester (-

-OR) of the carboxyl group or ester respectively with the N-terminal amino group to form a cyclic peptide. For example, after synthesis and cleavage to give the peptide acid, the free acid is converted in solution to an activated ester by an appropriate carboxyl group activator such as dicyclohexylcarbodiimide (DCC), *for example*, in methylene chloride (CH_2Cl_2), dimethyl formamide (DMF), or mixtures thereof. The cyclic peptide is then formed by displacement of the activated ester with the N-terminal amine. Cyclization, rather than polymerization, can be enhanced by use of very dilute solutions according to methods well known in the art.

Peptide mimetics as understood in the art and provided by the invention are structurally similar to the paradigm peptide encoded by each of the sense-oriented GSEs of the invention, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH_2NH--, --CH_2S--, --CH_2CH_2--, --CH=CH– (in both *cis* and *trans* conformers), --$COCH_2$--, --$CH(OH)CH_2$ --, and --CH_2SO--, by methods known in the art and further described in the following references: Spatola,1983, *in* CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS, (Weinstein, ed.), Marcel Dekker: New York, p. 267; Spatola, 1983, *Peptide Backbone Modifications* 1: 3; Morley, 1980, *Trends Pharm. Sci.* pp. 463-468; Hudson *et al.*, 1979, *Int. J. Pept. Prot. Res.* 14: 177-185; Spatola *et al.*, 1986, *Life Sci.* 38: 1243-1249; Hann, 1982, *J. Chem. Soc. Perkin Trans.* I 307-314; Almquist *et al.*, 1980, *J. Med. Chem.* 23: 1392-1398; Jennings-White *et al.*, 1982, *Tetrahedron Lett.* 23: 2533; Szelke *et al.*, 1982, European Patent Application, Publication No. EP045665A; Holladay *et al.*, 1983, *Tetrahedron Lett.* 24: 4401-4404; and Hruby, 1982, *Life Sci.* 31: 189-199, each of which is incorporated herein by reference. Such peptide mimetics may have significant advantages over polypeptide embodiments, including, for example: being more economical to produce, having greater chemical stability or enhanced pharmacological properties (such half-life, absorption, potency, efficacy, etc.), reduced antigenicity, and other properties.

Mimetic analogs of the tumor-inhibiting peptides of the invention may also be obtained using the principles of conventional or rational drug design (*see*, Andrews *et al.*, 1990, *Proc. Alfred Benzon Symp.* 28: 145-165; McPherson, 1990, *Eur. J. Biochem.* 189:1-24; Hol *et al.*, 1989a, *in* MOLECULAR RECOGNITION: CHEMICAL AND BIOCHEMICAL PROBLEMS, (Roberts, ed.); Royal Society of

Chemistry; pp. 84-93; Hol, 1989b, *Arzneim-Forsch.* 39:1016-1018; Hol, 1986, *Agnew Chem. Int. Ed. Engl.* 25: 767-778, the disclosures of which are herein incorporated by reference).

In accordance with the methods of conventional drug design, the desired mimetic molecules are obtained by randomly testing molecules whose structures have an attribute in common with the structure of a "native" peptide. The quantitative contribution that results from a change in a particular group of a binding molecule can be determined by measuring the biological activity of the putative mimetic in comparison with the tumor-inhibiting activity of the peptide. In a preferred embodiment of rational drug design, the mimetic is designed to share an attribute of the most stable three-dimensional conformation of the peptide. Thus, for example, the mimetic may be designed to possess chemical groups that are oriented in a way sufficient to cause ionic, hydrophobic, or van der Waals interactions that are similar to those exhibited by the tumor-inhibiting peptides of the invention, as disclosed herein.

The preferred method for performing rational mimetic design employs a computer system capable of forming a representation of the three-dimensional structure of the peptide, such as those exemplified by Hol, 1989a, *ibid.*; Hol, 1989b, *ibid.*; and Hol, 1986, *ibid.* Molecular structures of the peptido-, organo- and chemical mimetics of the peptides of the invention are produced according to those with skill in the art using computer-assisted design programs commercially available in the art. Examples of such programs include SYBYL 6.5®, HQSAR™, and ALCHEMY 2000™ (Tripos); GALAXY™ and AM2000™ (AM Technologies, Inc., San Antonio, TX); CATALYST™ and CERIUS™ (Molecular Simulations, Inc., San Diego, CA); CACHE PRODUCTS™, TSAR™, AMBER™, and CHEM-X™ (Oxford Molecular Products, Oxford, CA)and CHEMBUILDER3D™ (Interactive Simulations, Inc., San Diego, CA).

The peptido-, organo- and chemical mimetics produced using the peptides disclosed herein using, for example, art-recognized molecular modeling programs are produced using conventional chemical synthetic techniques, most preferably designed to accommodate high throughput screening, including combinatorial chemistry methods. Combinatorial methods useful in the production of the peptido-, organo- and chemical mimetics of the invention include phage display arrays, solid-phase synthesis and combinatorial chemistry arrays, as provided, for

example, by SIDDCO, Tuscon, Arizona; Tripos, Inc.; Calbiochem/Novabiochem, San Diego, CA; Symyx Technologies, Inc., Santa Clara, CA; Medichem Research, Inc., Lemont, IL; Pharm-Eco Laboratories, Inc., Bethlehem, PA; or N.V. Organon, Oss, Netherlands. Combinatorial chemistry production of the peptido-, organo- and chemical mimetics of the invention are produced according to methods known in the art, including but not limited to techniques disclosed in Terrett, 1998, COMBINATORIAL CHEMISTRY, Oxford University Press, London; Gallop *et al.*, 1994, "Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries," *J. Med. Chem.* 37: 1233-51; Gordon *et al.*, 1994, "Applications of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions," *J. Med. Chem.* 37: 1385-1401; Look *et al.*, 1996, *Bioorg. Med. Chem. Lett.* 6: 707-12; Ruhland *et al.*, 1996, *J. Amer. Chem. Soc.* 118: 253-4; Gordon *et al.*, 1996, *Acc.Chem. Res.* 29: 144-54; Thompson & Ellman, 1996, *Chem. Rev.* 96: 555-600; Fruchtel & Jung, 1996, *Angew. Chem. Int. Ed. Engl.* 35: 17-42; Pavia, 1995, "The Chemical Generation of Molecular Diversity", Network Science Center, www.netsci.org; Adnan *et al.*, 1995, "Solid Support Combinatorial Chemistry in Lead Discovery and SAR Optimization," *Id.*, Davies and Briant, 1995, "Combinatorial Chemistry Library Design using Pharmacophore Diversity," *Id.*, Pavia, 1996, "Chemically Generated Screening Libraries: Present and Future," *Id.*; and U.S. Patents, Nos. 5,880,972 to Horlbeck; 5,463,564 to Agrafiotis *et al.*; 5,331573 to Balaji *et al.*; and 5,573,905 to Lerner *et al.*

The invention also provides methods for using the genes identified herein (particularly the genes set forth in Table 3) to screen compounds to identify inhibitors of expression or activity of said genes. In the practice of this aspect of the methods of the invention, cells expressing a gene required for cell growth, particularly a gene identified in Table 3, are assayed in the presence and absence of a test compound, and test compounds that reduce expression or activity of the gene or gene product identified thereby. Additionally, the assays can be performed under suicide selection conditions, wherein compounds that inhibit cell growth by inhibiting expression or activity of the gene select for survival of the cells. In alternative embodiments, reporter gene constructs of the invention are used, wherein expression of the reporter gene is reduced in the presence but not the absence of the test compound.

The methods of the invention are useful for identifying compounds that inhibit the growth of tumor cells, most preferably human tumor cells. The invention also provides the identified compounds and methods for using the identified compounds to inhibit tumor cell, most preferably human tumor cell growth. Exemplary compounds include neutralizing antibodies that interfere with gene product activity; antisense oligonucleotides, developed either as GSEs according to the methods of the invention or identified by other methods known in the art; ribozymes; triple-helix oligonucleotides; and "small molecule" inhibitors of gene expression or activity, preferably said small molecules that specifically bind to the gene product or to regulatory elements responsible for mediating expression of a gene in Table 3.

The invention also provides embodiments of the compounds identified by the methods disclosed herein as pharmaceutical compositions. The pharmaceutical compositions of the present invention can be manufactured in a manner that is itself known, *e.g.*, by means of a conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus can be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

Non-toxic pharmaceutical salts include salts of acids such as hydrochloric, phosphoric, hydrobromic, sulfuric, sulfinic, formic, toluenesulfonic, methanesulfonic, nitric, benzoic, citric, tartaric, maleic, hydroiodic, alkanoic such as acetic, $HOOC-(CH_2)_n-CH_3$ where n is 0-4, and the like. Non-toxic pharmaceutical base addition salts include salts of bases such as sodium, potassium, calcium, ammonium, and the like. Those skilled in the art will recognize a wide variety of non-toxic pharmaceutically acceptable addition salts.

For injection, tumor cell growth-inhibiting compounds identified according to the methods of the invention can be formulated in appropriate aqueous solutions, such as physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal and transcutaneous

administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions can be used, which can optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers can be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions can take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds can be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions can contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension can also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use. The compounds can also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in

an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system can be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system can be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components can be varied: for example, other low-toxicity nonpolar surfactants can be used instead of polysorbate 80; the fraction size of polyethylene glycol can be varied; other biocompatible polymers can replace polyethylene glycol, *e.g.* polyvinyl pyrrolidone; and other sugars or polysaccharides can substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds can be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also can be employed, although usually at the cost of greater toxicity. Additionally, the compounds can be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules can, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein and nucleic acid stabilization can be employed.

The pharmaceutical compositions also can comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

The compounds of the invention can be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts can be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, phosphoric, hydrobromic, sulfinic, formic, toluenesulfonic, methanesulfonic, nitic, benzoic, citric, tartaric, maleic, hydroiodic, alkanoic such as acetic, $HOOC-(CH_2)_n-CH_3$ where n is 0-4, and the like. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. Non-toxic pharmaceutical base addition salts include salts of bases such as sodium, potassium, calcium, ammonium, and the like. Those skilled in the art will recognize a wide variety of non-toxic pharmaceutically acceptable addition salts.

Pharmaceutical compositions of the compounds of the present invention can be formulated and administered through a variety of means, including systemic, localized, or topical administration. Techniques for formulation and administration can be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA. The mode of administration can be selected to maximize delivery to a desired target site in the body. Suitable routes of administration can, for example, include oral, rectal, transmucosal, transcutaneous, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternatively, one can administer the compound in a local rather than systemic manner, for example, *via* injection of the compound directly into a specific tissue, often in a depot or sustained release formulation.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays, as disclosed herein. For example, a dose can be formulated in animal models to achieve a

circulating concentration range that includes the EC_{50} (effective dose for 50% increase) as determined in cell culture, *i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of bacterial cell growth. Such information can be used to more accurately determine useful doses in humans.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination, the severity of the particular disease undergoing therapy and the judgment of the prescribing physician.

Preferred compounds of the invention will have certain pharmacological properties. Such properties include, but are not limited to oral bioavailability, low toxicity, low serum protein binding and desirable *in vitro* and *in vivo* half-lives. Assays may be used to predict these desirable pharmacological properties. Assays used to predict bioavailability include transport across human intestinal cell monolayers, including Caco-2 cell monolayers. Serum protein binding may be predicted from albumin binding assays. Such assays are described in a review by Oravcová *et al.* (1996, *J. Chromat. B* 677: 1-27). Compound half-life is inversely proportional to the frequency of dosage of a compound. *In vitro* half-lives of compounds may be predicted from assays of microsomal half-life as described by Kuhnz and Gieschen (1998, DRUG METABOLISM AND DISPOSITION, Vol. 26, pp. 1120-1127).

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Compounds that exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage

can be chosen by the individual physician in view of the patient's condition. (*See, e.g.* Fingl *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch.1, p.1).

Dosage amount and interval can be adjusted individually to provide plasma levels of the active moiety that are sufficient to maintain tumor cell growth-inhibitory effects. Usual patient dosages for systemic administration range from 100 - 2000 mg/day. Stated in terms of patient body surface areas, usual dosages range from 50 - 910 mg/m^2/day. Usual average plasma levels should be maintained within 0.1-1000 μM. In cases of local administration or selective uptake, the effective local concentration of the compound cannot be related to plasma concentration.

The following Examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature.

EXAMPLES

1. Production of Normalized Tumor Library from MCF-7 Human Breast Cancer Cells

A normalized cDNA fragment library was generated from MCF-7 breast carcinoma cell line (estrogen receptor positive, wild-type for p53; ATCC Accession No. HTB22, American Type Culture Collection, Manassas, VA). Poly(A)+ RNA from MCF-7 cells was used to prepare a population of normalized cDNA fragments through a modification of the procedure described in Gudkov and Roninson (1997). Briefly, RNA was fragmented by heating at 100°C for 9 minutes. Double-stranded cDNA was generated from this heat-fragmented RNA using the Gibco Superscript kit with a reverse-transcription primer (5'-GGATCCTCACTCACTCANNNNNNNN-3'; SEQ ID NO. 1). This primer contains a random octamer sequence at its 3' end for random priming, and it carries a tag (termed "stop adaptor" in its double-stranded form) that provides TGA stop codons in all three open reading frames, together with *Bam*HI restriction site. PCR assays were used to establish the presence of β2-microglobulin, β-actin and estrogen receptor mRNA sequences in this cDNA preparation. Double-stranded cDNA fragments were ligated to the following adaptor:

5'GTACCTGAGTTATA**GGATCC**CT*GCC*ATG*CC*ATG*CC*ATG3' (SEQ ID NO. 2)

3'**CCTAGG**GAC*GGTACGGTACGGTAC* 5'(SEQ ID NO. 3)

The latter adaptor ("start adaptor") contains translation start sites in all three frames, together with a *Bam*HI site. The double-stranded cDNA was amplified by PCR with primers that anneal to the start and stop adaptors. Although the start adaptor is initially ligated at both ends of cDNA fragments, the PCR products were generated predominantly by the two different primers and contain the start adaptor only at the 5' but not at 3' end. This desirable outcome is explained by the "PCR suppression effect", due to PCR inhibition by panhandle-like structures formed upon renaturation of sequences flanked by an inverted repeat (Siebert *et al.*, 1995, *Nucleic Acids Res.* 23: 1087-1088). Furthermore, any residual start adaptors at the 3' ends were subsequently removed by *Bam*HI digestion prior to cloning. The amplified cDNA fragment population was again tested for the presence of β2-microglobulin, β-actin and estrogen receptor sequences. This procedure produced a population of randomly initiating and terminating double-stranded cDNA fragments (100-400 bp size), which are tagged by different adaptors at the ends corresponding to the 5' and 3' direction of the original mRNA. The 5' adaptor contains translation initiation codons in three open reading frames, and the 3' adaptor contains stop codons in all three reading frames. Such fragments direct the synthesis of peptides derived from the parental protein when cloned in sense orientation, or give rise to antisense RNA molecules when cloned in antisense orientation.

The cDNA fragment mixture was subjected to normalization, through a modification of the procedure of Patanjali *et al.* (1991, *Proc. Natl. Acad. Sci. USA* 88: 1943-1947), based on C_0t fractionation. Normalization was achieved by reannealing portions of denatured cDNA for 24, 48, 72, or 96 hours. Single-stranded products were separated from re-annealed double stranded DNA by hydroxyapatite chromatography. Normalization of cDNA fragments was tested by Southern hybridization with probes corresponding to genes expressed to different levels in MCF-7 cells and performed with each single-stranded fraction. This analysis indicated that the content of β-actin, an abundant mRNA species, decreased over normalization time, with the lowest content found at the 96 hr time point. Conversely, a moderately-abundant cDNA sequence, c-MYC and a low-abundant cDNA sequence, MDR1 (which was undetectable in MCF-7 cDNA prior

to normalization) increased their levels to those comparable with β-actin by 96 hr, suggesting that the 96 hr fraction was the best-normalized. To confirm the normalization of the 96 hr fraction, this DNA was digested (on a small scale) with *Bam*HI, ligated into a plasmid vector and transformed into *E. coli* (Top10) by electroporation. Colony hybridization analysis was performed on nitrocellulose filters to which 10,000 colonies were plated, using radiolabeled probes for different genes. The following signal numbers per filter were obtained: β-actin, 3 signals; MDR1, 3 signals; C-MYC, 2 signals; C-FOS, 2 signals. These results indicated that the sequences from the tested genes are found on average in 1 of 3,000-5,000 clones of this library, and also confirmed that the 96 hr fraction was normalized.

The normalized cDNA fraction was amplified by PCR and ligated into IPTG-inducible retroviral vector LNXCO3 (Chang and Roninson, 1996, *Gene* 183: 137-142). The ligation produced a library of approximately 50 million clones. Percent recombination in this library was assessed by PCR of the DNA from bacterial colonies, using primers that flank the insertion site of LNXCO3. The number of clones containing an insert was 131/150 or 87%. Most of the inserts ranged in size from 100 to 300 bp. For further characterization of the library, a fraction of the inserts were recloned into the pcDNA3 vector. The insert sequences of 69 randomly picked clones in pcDNA3 were determined using a high-throughput DNA sequencer, and analyzed for homology to known gene sequences in the public-domain database. Fifty-two of the inserts matched no known genes, 16 corresponded to different human genes, and one sequence was found to be of bacterial origin. This normalized MCF-7 cDNA fragment library was used to select growth-inhibitory GSEs in breast carcinoma cells.

2. Production of breast cancer recipient cells

The normalized tumor library described in Example 1 was prepared from MCF-7 human breast carcinoma cells. As recipient cells for GSE selection, a different breast carcinoma cell line, MDA-MB-231 (ATCC Accession No. HTB26) was chosen. This line represents a more malignant class of breast cancers relative to MCF-7: it is estrogen receptor-negative and p53-deficient. The choice of different cell lines as the source of RNA and as the recipient was aimed at isolating

growth-inhibitory GSEs that are more likely to be effective against different types of breast cancer.

MDA-MB231 cells were first rendered susceptible to infection with ecotropic retroviruses, which can be readily generated at a high titer using convenient packaging cell lines, and are not infectious to humans or unmodified human cells. MDA-MB-231 cells were infected with amphotropic recombinant virus that carries the gene for the murine ecotropic receptor in retroviral vector LXIHis (Levenson *et al.*, 1998, *Hum. Gene Ther.* 9: 1233-1236), and the infected cell population was selected with histidinol. The susceptibility of the selected cells to infection with ecotropic retroviruses was determined by infecting such cells with an ecotropic retrovirus LXSE (Kandel *et al.*, 1997, *Id.*) that carries the gene for the Green Fluorescent Protein (GFP). Over 86% of LXSE-infected cells were positive for GFP fluorescence (as determined by flow cytometry), indicating a correspondingly high infection rate. These cells were next transfected with the 3'SS plasmid (Stratagene) that carries the *Lac*I repressor (Fieck *et al.*, 1992, *Nucleic Acids Res.* 20: 1785-1791) and the hygromycin resistance marker, and stable transfectants were selected with hygromycin. The selected transfectants were subcloned, and 33 single-cell clones were individually tested for IPTG-regulated expression of a LacI-inhibited promoter. This testing was carried out by transient transfection of the cell clones with pCMVI3luc plasmid (Stratagene) that expresses luciferase from the LacI-regulated CMV promoter. As a positive control, the same assay was carried out on a previously characterized well-regulated fibrosarcoma cell line HT1080 3'SS6 (Chang and Roninson, 1996, *Id.*; Chang *et al.*, 1999, *Id.*). Three of the tested clones showed the induction of luciferase expression in the presence of IPTG at a level similar to that of HT1080 3'SS6.

These clones were further tested by the following assays. The first assay was infection with LXSE ecotropic retrovirus, followed by FACS analysis of GFP fluorescence, to determine the susceptibility to ecotropic infection. The second assay was ecotropic retroviral transduction with IPTG-regulated retrovirus LNLucCO3 (Chang and Roninson, 1996), followed by G418 selection and testing for IPTG inducibility of luciferase expression. The third assay was the infection with IPTG-regulated ecotropic retrovirus LNp21CO3 (Chang *et al.*, 1999, *Id.*), which carries the cell cycle inhibitor p21 (a positive control for an IPTG-inducible

genetic inhibitor), followed by BrdU suicide selection (described below) in the presence and in the absence of IPTG. Based on the results of these assays, a cell line called MDA-MB231 3'SS31 was selected as being optimal for growth-inhibitory GSE selection. This cell line showed about 80% infectability with ecotropic retroviruses, approximately10-fold inducibility by IPTG (which is higher than the concurrently determined value for HT1080 3'SS6) and over 20-fold increase in clonogenic survival of BrdU suicide upon infection with LNp21CO3.

3. Isolation of Tumor Cell Growth Inhibiting Genetic Suppressor Elements

The MCF-7 derived normalized tumor library in the LNXCO3 vector was transduced into MDA-MB231 3'SS31 cell line by ecotropic retroviral transduction using the BOSC23 packaging cell line (Pear *et al.*, 1993, *Id.*), as described in Roninson *et al.* (1998, *Methods Enzymol.* 292: 225-248). Two hundred million (2 x 10^8) recipient cells were infected and selected with G418. The infection rate (as determined by the frequency of G418-resistant colonies) was 36%. Eighty million ($8x10^7$) G418-selected infectants were subjected to selection for IPTG-dependent resistance to BrdU suicide, as follows. Cells were plated at 10^6 cells per P150 and treated with 50 μM IPTG for 36 hrs, then with 50 μM IPTG and 50 μM BrdU for 48 hrs. Cells were thereafter incubated with 10 μM Hoechst 33342 for 3 hrs and illuminated with fluorescent white light for 15 min on a light box, to destroy the cells that grew and incorporated BrdU in the presence of IPTG. Cells were then washed twice with phosphate-buffered saline and allowed to recover in G418-containing medium without IPTG or BrdU for 7-10 days. The surviving cells were then subjected to a second step of BrdU selection under the same conditions. Control plates were selected in the absence of IPTG, and representative plates were stained to count the colonies; these results are shown in Figure 4. The number of surviving colonies after the second step of selection in the presence of IPTG was approximately three times higher than the corresponding number in the absence of IPTG. In contrast, control cells infected with an insert-free LNXCO3 vector showed no difference in BrdU survival in the presence or in the absence of IPTG. As a positive control, cells were infected with p21-expressing LNp21CO3, but the number of survivors in the presence of IPTG was too high to count. These results demonstrated that the frequency of library-infected cells that survived BrdU

suicide selection increased in IPTG-dependent manner, consistent with successful selection of IPTG-inducible growth-inhibitory GSEs.

Genomic DNA was isolated from the two-step selected library-transduced cells and used as a template for PCR, using vector-derived sequences flanking the inserts as primers. The PCR-amplified mixture of inserts from the selected cells was recloned into LNXCO3 vector and close to 3,000 randomly picked plasmid clones from the library of selected fragments were sequenced by high-throughput DNA sequencing by PPD Discovery, Inc., Menlo Park, CA. 1482 clones containing human cDNA fragments were identified among these sequences by BLAST homology search using the NCBI database and analyzed to identify genes that gave rise to the selected cDNA fragments. Ninety-three genes were found to give rise to two or more of the sequenced clones, indicating the enrichment for such genes in the selected library, with 67 genes represented by three or more clones. Forty-nine of the enriched genes were represented by two or more non-identical sequences. The sequences of the enriched clones are provided in Table 4 and the Sequence Listing. Many of these clones encode peptides derived from the corresponding gene products. The sequences of these growth-inhibitory peptides are provided in Table 5 and in the Sequence Listing as SEQ ID NOS. 229-314. The enriched genes with the corresponding accession numbers, as well as the numbers of selected clones and different sequences derived from each genes are listed in Table 1. Table 2 lists enriched genes previously known to be involved in cell proliferation, and Table 3 lists enriched genes that were not previously known to be involved in cell proliferation.

The following criteria were used for assigning genes to Table 2 or Table 3. The function of each gene was first confirmed according to the corresponding entry in the LocusLink database of NCBI. On the basis of this information, genes that are essential for basic cell functions (such as general transcription or translation), and genes known to play a role in cell cycle progression or carcinogenesis were excluded from Table 3 and assigned to Table 2. The functions of the other genes were then investigated through a database search of the art, using all the common names of the gene listed in LocusLink as keywords for the search. Through this analysis, additional genes were assigned to Table 2 by the following criteria (i) if overexpression of the gene, alone or in combinations, was shown to promote neoplastic transformation or cell immortalization; (ii) if

inhibition of the gene function or expression was shown to produce cell growth inhibition or cell death; (iii) if homozygous knockout of the gene was shown to be embryonic lethal in mammals; or (iv) if the gene was found to be activated through genetic changes (such as gene amplification, rearrangement or point mutations) in a substantive fraction of any type of cancers. Genes that did not satisfy any of the above criteria were then assigned to Table 3.

4. Analysis of Tumor Cell Growth Inhibiting Genetic Suppressor Elements

Individual selected clones representative of enriched genes have been analyzed by functional testing for GSE activity. Results of these assays are summarized in Table 1. The principal assay involves the transduction of individual putative GSE clones (in the LNXCO3 vector) into MDA-MB-231-3'SS31 cells, followed by G418 selection of infected populations (for the *neo* gene of LNXCO3) and testing the transduced populations for IPTG-dependent survival of BrdU suicide. The latter assay was carried out as follows. Infected cells (200,000 per P100, in triplicate) were treated with 50 μM IPTG for 72 hrs, then with 50 μM IPTG and 50 μM BrdU for 48 hrs. A parallel set of cells was treated in the same way but without IPTG (in triplicate). Cells were then illuminated with white light and allowed to recover in the absence of BrdU and IPTG for 12-14 days. Results are expressed as the average number of colonies per P100, with standard deviations. In each set of assays, insert-free LNXCO3 vector was used as a negative control. As a positive control, LNXCO3 vector expressing CDK inhibitor p21 was used, but this control consistently gave excessively positive values of surviving colonies. Alternative positive controls comprised a GSE derived from a proliferation-associated transcription factor Stat3, which produced moderate but reproducibly positive results in multiple assays. Table 1 lists the results of this assay (IPTG-dependent survival of BrdU suicide) as positive ("A" in Functional Assays column) if *t*-test analysis of the difference in the number of colonies surviving in the presence and in the absence of IPTG provides a significance value of $P<0.05$. Results of this analysis on a subset of positive GSEs are shown in Figure 5.

The assay for IPTG-dependent survival of BrdU suicide was performed for GSEs derived from 38 genes with positive results. Several infected cell

populations that scored positive in this assay were also tested by a more stringent assay for direct growth inhibition by IPTG. None of the tested populations, however, showed significant growth inhibition by IPTG. A similar result (positivity in BrdU selection but not in the growth inhibition assay) was reported by Pestov *et al.* (1998, *Id.*) for a weak growth-inhibitory cDNA clone encoding a ubiquitin-conjugation enzyme. To determine whether increased BrdU survival in such cell populations reflects the heterogeneity of GSE expression and function among the infected cells, multiple (10 or more) clonal cell lines were generated from a subset of infected populations and tested for the ability to be growth-inhibited by IPTG. Through this process, IPTG-inhibited cell lines containing GSEs from 19 of the enriched genes were produced. The genes that scored positive by this assay are indicated in Table 1 ("B" in Functional Assays column). In contrast to these GSE-containing cell lines, cells transduced with an insert-free LNXCO3 vector showed no growth inhibition in the presence of IPTG. Results of IPTG growth inhibition assays with positive cell lines are shown in Figure 6.

Putative GSEs from 7 of the tested genes gave a greatly diminished yield of G418-resistant infectants, relative to cells infected with the control LNXCO3 virus or with other tested clones. When the resulting small populations of G418-resistant cells infected with these clones were expanded and tested for IPTG-dependent survival of BrdU suicide, almost all of these populations produced negative results. Remarkably, most of the genes in this category ("C" in Functional Assays column of Table 1) are known to be important positive regulators of cell growth (JUN B, INT-2, MCM-3 replication protein, delta and eta isoforms of protein kinase C) and therefore are expected to give rise to growth-inhibitory GSEs. Since LNXCO3 vector is known to provide substantial basal expression in the absence of IPTG (Chang and Roninson, 1996), it seems likely that this group may include the strongest functional GSEs, which inhibit cell growth even in the absence of IPTG. Altogether, GSEs from a total of 51 genes have so far been confirmed by functional assays (IPTG-dependent survival of BrdU suicide or IPTG-dependent growth inhibition) or a putative positive criterion (decreased apparent infection rate).

The genes shown in Table 2 are known to be positive regulators of the cell growth or neoplastic transformation. These include genes directly involved in cell cycle progression (such as CCN D1 and CDK2) or DNA replication (*e.g.* PCNA,

RPA3 or MCM-3), growth factors (*e.g.* INT-2/FGF-3 and TDGF1) and growth factor receptors (*e.g.* FGFR1, C-KIT), transcription factors known to be positive regulators of cell proliferation (*e.g.* STAT3, c-FOS, NF□B-1), several proliferation-associated signal transduction proteins, such as three isoforms of PKC (the primary target of tumor promoters) and three integrin proteins, as well as several ribosomal components required for protein synthesis. The enriched genes include many known protooncogenes, such as JunB and c-FOS (which gave rise to two of three growth-inhibitory GSEs isolated by Pestov and Lau (1994, *Id.*) from a 19-gene library in NIH 3T3 cells), a FOS-related gene, INT-2, c-KIT, LYN B (YES protooncogene), MET, RAN (a member of RAS family), several growth-promoting genes that are known to be amplified in cancers (CCN D1, CDK2, FGFR1), and several genes reported to be overexpressed in cancers. Some of the enriched genes have specific associations with breast cancer, including INT-2, originally identified as a mammary oncogene (Peters *et al.*, 1984, *Nature* 309: 273-275), CCN D1 and FGFR1 found to be amplified in a substantial minority of breast cancers (Barnes and Gillett, 1998, Breast Cancer Res Treat. 52: 1-15; Jacquemier *et al.*, 1994, *Int. J. Cancer* 59: 373-378), and HSPCA, which was shown to be expressed in all the tested breast cancers (143 total) at a higher level than in non-malignant breast tissue (Jameel *et al.*, 1992, *Int. J. Cancer* 50: 409-415). The abundance of such genes among the selected sequences provides strong validation of this approach to the elucidation of positive growth regulators in breast carcinoma cells.

The genes in Table 3 have no known function in growth regulation. These genes encode several transcription factors, proteins involved in signal transduction or cell adhesion, a number of proteins involved in RNA transport or protein trafficking and processing, a group of genes with miscellaneous other functions that are not related to cell growth, and 10 genes, the functions of which are presently unknown.

Of special interest, at least three of the genes in Table 3 appear to be inessential for growth of normal cells, since homozygous knockout of these genes in mice does not prevent the development of adult animals (except for some limited developmental abnormalities). These genes include L1CAM (Dahme *et al.*, 1997, *Nat. Genet.* 17 346-349), ICAM2 (Gerwin *et al.*, 1999, *Immunity* 10: 9-19), and von Willebrand factor (Denis *et al.*, 1998, *Proc Natl Acad Sci USA* 95: 9524-

9529). The effect of GSEs derived from these genes on breast carcinoma cells suggests that inhibition of such "inessential" genes may have a desirable tumor-specific or tissue-specific antiproliferative effect.

A striking example of an apparently inessential gene enriched in the selected library, which has been independently identified as a highly promising target for breast cancer treatment, is provided by HSPCA (included in Table 2). The basic function of this gene, which belongs to of a heat shock responsive family of chaperone proteins, which play a role in refolding of mature proteins, does not indicate that it should be required for cell growth. HSPCA, however, was found to play a role in stabilizing several proteins that are involved in oncogenic pathways, including Raf, Met, steroid receptors, and members of the HER kinase family, and to serve as the target of an antitumor antibiotic geldanamycin (Stebbins *et al.*, 1997, *Cell* 89: 239-250). The HSPCA-inhibiting geldanamycin analog 17-AAG has been shown to arrest the growth of breast carcinoma cell lines (including MDA-MB-231; Munster et al, 2001, *Cancer Res.* 61: 2945-2952) and to sensitize such cells to chemotherapy-induced apoptosis (Munster *et al.*, 2001, *Clin Cancer Res* 7: 2228-2236); 17-AAG is currently in clinical trial. The example of HSPCA suggests that other apparently inessential genes identified by GSE selection are likely to provide similarly promising targets for cancer treatment. Some of these potential novel targets are described in more detail in the next section.

5. Potential novel drug targets.

Several of the selected genes warrant consideration as potential novel targets for cancer drug development. Non-limiting examples are as follows.

L1CAM. L1 cell adhesion molecule (L1CAM) is represented in the set of growth-inhibiting GSEs by eight sense-oriented and four antisense-oriented GSEs. L1CAM is a 200-220 kDa type I membrane glycoprotein of the immunoglobulin superfamily expressed in neural, hematopoietic and certain epithelial cells. The non-neuronal (shortened) form of L1CAM is expressed highly in melanoma, neuroblastoma, and other tumor cell types, including breast. L1CAM is found not only in membrane-bound form but also in the extracellular matrix of brain and tumor cells. Soluble L1CAM directs the migration of glioma cells, and one of anti-L1CAM antibodies was found to inhibit this migration (Izumoto *et al.*, 1996,

Cancer Res. 56: 1440-1444). Such an antibody might be useful as an initial prototype agent to validate L1CAM as a cancer drug target.

As a cell surface molecule, L1CAM should be easily accessible to different types of drugs. Figures 7A and 7B illustrate morphological effects of an L1CAM-derived GSE in a clonal IPTG-inhibited cell line. Four-day treatment with IPTG drastically altered cell morphology, with the cells developing lamellipodia and apparent focal adhesion plaques (Fig. 7A). This effect suggests that the IPTG-induced GSE affects cell adhesion, as would have been expected from targeting L1CAM. GSE induction not only arrested cell growth but also induced mitotic catastrophe in 15-20% of IPTG-treated cells. Mitotic catastrophe is a major form of tumor cell death (Chang *et al.*, 1999, *Id.*), which is characterized by abnormal mitotic figures and formation of cells with multiple micronuclei (Fig. 7B). The ability of a GSE to induce mitotic catastrophe is a good general indication for the potential promise of a GSE-inhibited target.

Human L1CAM gene is mutated in patients with a severe X-linked neurological syndrome (CRASH: corpus callosum hypoplasia, retardation, aphasia, spastic paraplegia and hydrocephalus). L1CAM "knockout" (-/-) mice develop to adulthood and appear superficially normal (slightly smaller than adults), but they have a shortened lifespan due to CRASH-like neurological deficits, which may be related to a decrease in neurite outgrowth (Dahme *et al.*, 1997, *Id.*). These observations suggest that targeting L1CAM in an adult cancer patient should not have major toxicity outside of the nervous system, where most drugs will not penetrate due to the blood-brain barrier. Furthermore, it is quite likely that the neurological effects result only from a lack of L1CAM during embryonic development and would not develop from L1CAM inhibition in an adult.

ICAM2. The intercellular cell adhesion molecule-2 (ICAM2) is represented in the set of growth-inhibiting GSEs by two sense-oriented and one antisense-oriented GSE. ICAM2 has many similarities to L1CAM and is also inessential for the growth of normal cells (Gerwin *et al.*, 1999, *Id.*). Anti-ICAM2 antibodies, for example, are attractive possibilities for prototype drugs.

NIN283. This gene has recently been described (Araki *et al.*, 2001, *J. Biol. Chem.* 276: 34131-34141) as being induced in Schwann cells upon **n**erve **in**jury and termed NIN283. Induction of NIN283 is a part of injury response of Schwann cells, which then act to promote the growth of the injured nerve. NIN283 is also

induced by nerve growth factor (NGF). Like L1CAM, NIN283 is expressed primarily in the brain. It is localized to lysosomes, is highly conserved in evolution (with identifiable homologs in *Drosophila* and *C. ele*gans), and contains a unique combination of a single zinc finger and a RING finger motif. Based on these structural features and localization, Araki *et al.* (2001, *Id.*) speculated that NIN283 may be involved in ubiquitin-mediated protein modification and degradation. With this putative function in protein modification, stress inducibility and evolutionary conservation, NIN283 appears analogous to the above-discussed HSPCA.

Here, this gene was found to give rise to one of the strongest functionally active GSEs in breast carcinoma growth-inhibition assays. The available information on functional domains of NIN283 should be useful in structure-based rational design of small molecule inhibitors of this interesting protein.

ATF4. Activating transcription factor 4 gave rise to the most highly enriched antisense GSE in these selection assays. Homozygous knockout of ATF4 results in only minor developmental abnormalities (in the eye lens; Tanaka *et al.*, 1998, *Genes Cells* 3: 801-810; Hettmann *et al.*, 2000, *Dev. Biol.* 222: 110-123), indicating that this factor is not essential for normal cell growth. The results disclosed herein implicate ATF4 in breast cancer cell proliferation and are strengthened by reports in the art that ATF4 expression and function are augmented by heregulin β1, a factor that stimulates the growth of breast cancer cells (Talukder *et al.*, 2000, *Cancer Res.* 60: 276-281).

Zinedin. Zinedin is a recently described calmodulin-binding protein with a WD repeat domain, which is preferentially expressed in the brain (Castets *et al.*, 2000, *J. Biol. Chem.* 275: 19970-19977). This expression pattern suggests that zinedin-targeting drugs are unlikely to have an effect on any normal proliferating cells. An antisense-oriented GSE derived from zinedin, however, was found herein to inhibit breast carcinoma cell growth, both by the IPTG-dependent BrdU suicide assay and by the ability to give rise to an IPTG-inhibited cell line. Structural analysis of zinedin indicates specific domains that apparently mediate its interactions with calmodulin and caveolin (Castets *et al.*, *Id.*). Structure-based targeting of these domains, as well as screening based on the interference with

zinedin-calmodulin interactions, can be used as strategies for developing zinedin-targeting drugs.

Novel genes. Several genes identified by this selection have no known function, no significant homologies with known genes or identifiable functional domains. These results provide the first functional evidence for such genes. One of the most highly enriched and functionally active GSEs is designated GBC-1 (Growth of Breast Carcinoma 1). Translated protein sequence of GBC-1 matches a partial sense-oriented sequence of a hypothetical unnamed protein (accession No. XP_031920). GBC-1 GSE encodes a helical-repeat peptide. The strong growth-inhibitory activity of this GSE suggests that molecules derived from or mimicking this peptide are likely to have antitumor activity. The GBC-1 peptide disclosed herein can be regarded as a prototype drug, the structure of which can be used to direct rational design of a synthetic compound.

Among other novel genes identified in the instant invention, two genes, designated herein GBC-3 (Growth of Breast Carcinoma 3) and GBC-11 (Growth of Breast Carcinoma 11) are the most highly enriched, and their GSEs show strong functional activity. Cell lines that comprise these GSE and that are efficiently growth-inhibited by treatment with IPTG are useful for characterizing the cellular effects of GBC-3 or GBC-11 inhibition. GBC-3 matches an otherwise uncharacterized EST AA443027 and maps to chromosome 3q29, GBC-11 maps to chromosome 14 and does not match any known cDNA sequences. GBC-3 appears according to "Virtual Northern" analysis carried out using the NCBI SAGE database (http://www.ncbi.nlm.nih.gov/SAGE/_sagevn.cgi) to be expressed at a very low level in all cell types, suggesting that it may be an easy target to inhibit.

6. *In vivo* testing of test compounds

The efficacy of inhibiting expression or activity of the genes set forth in Table 3 is tested *in vivo* as follows.

Cells (1-2 x 10^6) expressing an IPTG-inducible GSE of the invention that inhibits expression or activity of a gene in Table 3 are injected into a mouse as a xenograft, most preferably in one flank of the mouse so that tumor growth can be visually monitored. IPTG-regulated gene expression in mouse xenografts of MDA-MB-231 breast carcinoma has been demonstrated in the art, for example by

Lee *et al.* (1997, *Biotechniques* 23: 1062-1068) and the experiments described herein can be performed substantially as described by Lee *et al.* but using the GSE-containing tumor cells of the invention. Conveniently, GSE-naïve tumor cells are injected in the opposite flank in each mouse. Two sets of injected mice are housed and maintained in parallel, with one set of mice having feed supplemented with IPTG at a concentration as taught by Lee *et al.* and the other set of mice not receiving IPTG supplemented food. Emergent tumors are observed on the mice under humane animal care conditions until the extent of tumor cell growth is life-threatening or inhumane. Biopsy samples are taken and the tumors measured and weighed after animal sacrifice to determine differences between the GSE-expressing and non-GSE-expressing tumors in each mouse and between mice fed IPTG and mice without IPTG supplementation.

IPTG-fed mice will bear one tumor of naïve xenograft cells whose growth is unaffected by IPTG. These tumors will be substantially identical to the size of both naïve xenograft cell and GSE-containing xenograft cell tumor in mice not fed IPTG. In contrast, the tumor produced from the GSE-containing xenograft cells in mice fed IPTG will be substantially smaller than the other tumors. Biopsy will show proliferating tumor cells in both naïve xenograft cell and GSE-containing xenograft cell tumor in mice not fed IPTG and naïve xenograft cells from IPTG-fed mice, and quiescent or dying cells in the GSE-containing xenograft tumor.

These results demonstrate that inhibition of expression or activity of genes set forth in Table 3 inhibits tumor cell growth in vivo.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

Table 1. Genes Enriched among 1482 Sequences of Clones Containing cDNA Inserts in the Selected Library

Gene	Accession #	#Sequences (s/as)	# clones	Functional Assays*
ATF4	NM_001675.1	5(as)	369	A
STAT5b	NM_012448.1	4(s), 4(as)	152	A,B
GBC-1	NM_031221.1	2 (s)	70	A,B
ARHG	NM_001665.1	5(s), 1(as)	43	A
VWF	NM_000552.2	6(s), 5(as)	39	B
MCM3	NM_002388.2	3(s), 4(as)	38	C
18S RNA	K03432.1	8(s), 4(as)	33	A
ITGB5	NM_002213.1	4(s), 1(as)	30	A,B
HSPCA	NM_005348.1	2(s)	27	B
STAT3	NM_003150.1	4(s), 3(as)	25	A,B
L1CAM	NM_000425.2	8(s), 4(as)	20	A,B
28S RNA	M27830.1	3 (s)	17	A
C-FOS	NM_005252.2	3(s), 3(as)	17	A
C-KIT	NM_021099.2	4(s), 2(as)	12	A
FEN1	NM_004111.3	2(s), 2(as)	12	A
GBC-3	AA443027	1(s)	12	A,B
NIN283	NM_032268	1(s)	11	A
ADPRT	NM_001618	1(s), 1(as)	10	
CCN D1	NM_001758.1	2(s), 2(as)	9	A
CDC20	NM_001255	1(as)	9	B
EFNA1	NM_004428	1(s), 3(as)	9	A
KIAA1270	XM_044835	1(as)	9	A
RPL31	NM_013403.1	2(s)	9	A,B
7SL	X04248.1	4(s), 1(as)	8	C
ENO1	NM_001428	2(s)	8	
GSTP	NM_000852	2(s)	8	
ICAM2	NM_000873	2(s), 1(as)	8	
INT-2/FGF3	NM_005247	2(s)	8	C
LYN	NM_002350	2(as)	8	A
RPS24	NM_001026	1(s), 1(as)	8	
FGFR1	NM_000604.2	2(s), 1(as)	6	A
HES6	XM_043579	1(s)	6	B
PKC zeta	NM_002744	2(s), 1(as)	6	B
RAN	NM_006325	1(s)	6	
RPA3	NM_002947.1	1(s)	6	A
ZIN	NM_013403.1	1(as)	6	A, B
TAF7	NM_005642	1(s)	6	A
AP1B1/BAM22	NM_001127.1	2(s)	5	A
HNRPF	NM_004966	1(s)	5	A
HNRPMT	AF222689	1(s)	5	A
NFkB-1	NM_003998.1	1(as)	5	A,B
NR3C1	NM_000176	1(s)	5	A
PKC delta	NM_006254.1	2(s), 1(as)	5	C
BAG-1	NM_004323.2	2(s)	4	A
GBC-11	W84777	1(s)	4	A,B
HNRPA2B1	NM_002137	1(s)	4	A
IF1	NM_016311.1	1(s)	4	A
ITGA4	NM_000885	1(s), 1(as)	4	
JunB	NM_002229.1	1(s)	4	C
GRP58	NM_005313.1	1(s), 1(as)	4	
PKC eta	NM_006255.1	3(s), 1(as)	4	A,B,C

Gene	Accession #	#Sequences (s/as)	# clones	Functional Assays*
PSMB7	NM_002799	1(s)	4	
RAB2L	NM_004761	1(s)	4	
RPL35	NM_004632.1	2(as)	4	C
CDK2	NM_001798.1	2(s)	3	A
DAP-3	NM_004632.1	2(as)	3	A,B
EIF-3	NM_003750	3(s)	3	A
GBC-12		1(s)	3	A
IGF2R	NM_000876	2(s)	3	
KIFC1	XM_042626	1(as)	3	
MET	NM_031517	2(s), 1(as)	3	
PCNA	NM_002592	1(s)	3	
PPP2R1B	NM_002716	2(as)	3	
RAB5B	NM_002868.1	1(s), 1(as)	3	
TDGF1	NM_003212	1(as)	3	
ARFAPTIN1	NM_014447	1(as)	2	
CDK10	NM_003674	2(s)	2	B
CREB1	NM_004379	1(s)	2	
EDF-1	NM_003792	1(s)	2	
FLJ10006	XM_041928	1(as)	2	
FLJ13052	NM_023018	1(s)	2	
FOSL2	NM_005253.1	1(s), 1(as)	2	
GBC-13		1(s)	2	
GBC-14	AL557138	1(s)	2	
GBC-15	BE079876	1(s)	2	
GBC-16		1(s)	2	
GBC-17		1(s)	2	
GBC-18		1(s)	2	
GNAS	M21139	1(as)	2	
IL4R	NM_000418	1(as)	2	
ITGA3	NM_002204	1(as)	2	
MAP2K2	NM_002755	2(as)	2	
MBD-1	NM_015847	1(s), 1(as)	2	B
MCM-6	NM_005915	1(s)	2	
MYL6	NM_021019	2(s)	2	A
NUMA1	NM_006185	1(s)	2	
PC4	NM_006713	1(s)	2	
RAD23A	NM_005053	1(s)	2	
REL	NM_002908	1(s)	2	
RPA1	NM_002945	1(as)	2	
RPL12	NM_000976	1(s)	2	
RPS29	NM_001032	1(s)	2	
SQSTM1	NM_003900	1(s)	2	

*A, confirmed by BrdU suicide assay; B, gave rise to cell line inhibited by IPTG; C, low infection rate

Table 2. Enriched Genes Previously Implicated in Cell Proliferation

Gene	Accession No.	#Sequences (s/as)	# clones	Description	Association with cancer
CCN D1	NM_001758	2(s), 2(as)	9	Cyclin, G1/S transition	Amplified in cancers
CDK2	NM_001798	2(s)	3	Cyclin-dependent kinase, S-phase	Amplified in cancers
PCNA	NM_002592	1(s)	3	DNA replication	Upregulated in cancers
RPA3	NM_002947	1(s)	6	DNA replication, excision repair	
RPA1	NM_002945	1(as)	2	DNA replication	
MCM3	NM_002388	3(s), 4(as)	38	DNA replication	
MCM6	NM_005915	1(s)	2	DNA replication	
FEN1	NM_004111	2(s), 2(as)	12	DNA replication and repair	
CDC20	NM_001255	1(as)	9	CDC2-related kinase, mitosis	
NUMA1	NM_006185	1(s)	2	Nuclear reassembly in late mitosis	
RAN	NM_006325	1(s)	6	Small GTPase, mitosis	Ras family
CDK10	NM_003674	2(s)	2	Cell cycle, G2/M	
C-KIT	NM_021099	4(s), 2(as)	12	Growth factor receptor, oncogene	Protooncogene
EFN A1	NM_004428	1(s), 3(as)	9	Receptor tyrosine kinase ligand	RAS pathway regulator
LYN	NM_002350	2(as)	8	Tyrosine kinase	YES protooncogene
INT-2/FGF-3	NM_005247	2(s)	8	Fibroblast growth factor	Mammary oncogene
FGFR1	NM_000604	2(s), 1(as)	6	Fibroblast growth factor receptor, tyrosine kinase	Amplified in breast cancers
IGF2R	NM_000876	2(s)	3	Insulin-like growth factor 2 receptor	Mutated in breast cancers
TDGF1	NM_003212	1(as)	3	Teratocarcinoma derived growth factor 1 (EGF family)	Overexpressed in teratocarcinomas
MET	NM_031517	2(s), 1(as)	3	Hepatocyte growth factor receptor	Protooncogene
IL4R	NM_000418	1(as)	2	Interleukin-4 receptor	
STAT3	NM_003150	4(s), 3(as)	25	Transcription factor	Upregulated in breast ca

Gene	Accession No.	#Sequences (s/as)	# clones	Description	Association with cancer
				(proliferation)	
STAT5b	NM_012448	4(s), 4(as)	152	Transcription factor (proliferation)	
C-FOS	NM_005252	3(s), 3(as)	17	AP-1 component	Protooncogene
NFκB-1	NM_003998	1(as)	5	Stress, apoptosis, paracrine activities	
TAF7	NM_005642	1(s)	6	Transcription initiation factor	
PC4	NM_006713	1(s)	2	General positive coactivator of transcription	
CREB1	NM_004379	1(s)	2	Transcription factor, regulates expression of cAMP-inducible genes including Cyclin A	
JUNB	NM_002229	1(s)	4	AP-1 component	Protooncogene
FOSL2	NM_005253	1(s), 1(as)	2	AP-1 component	FOS-related
REL	NM_002908	1(s)	2	Transcription factor	Protooncogene
ADPRT	NM_001618	1(s), 1(as)	10	Poly (ADP ribosyl) transferase	
PKC zeta	NM_002744	2(s), 1(as)	6	Serine/threonine protein kinase	Stimulated by tumor promoters
PKC delta	NM_006254	2(s), 1(as)	5	Serine/threonine protein kinase	Stimulated by tumor promoters
PKC eta	NM_006255	3(s), 1(as)	4	Serine/threonine protein kinase	Stimulated by tumor promoters
MAP2K2	NM_002755	2(as)	2	MAP kinase kinase	Implicated in medulloblastoma metastasis
GRP58	NM_005313	1(s), 1(as)	4	Membrane signal transduction	
PPP2R1B	NM_002716	2(as)	3	Protein phosphatase 2 regulatory subunit β	
BAG1	NM_004323	2(s)	4	Apoptosis inhibitor (Bcl-2 family)	Overexpressed in cancers
DAP3	NM_004632	2(as)	3	Positive/negative apoptosis regulator	Overexpressed in gliomas
ITGA4	NM_000885	1(s), 1(as)	4	Cell adhesion, signal transduction	Involved in Src pathway
ITGA3	NM_002204	1(as)	2	Cell adhesion, signal	Involved in colorectal cancer growth

Gene	Accession No.	#Sequences (s/as)	# clones	Description	Association with cancer
				transduction	
ITGB5	NM_002213	4(s), 1(as)	30	Cell adhesion, signal transduction	Correlates with invasiveness in gastric ca
AHRG	NM_001665.	5(s), 1(as)	43	Small GTPase, cytoskeletal reorganization	Ras family, contributes to Ras transforming activity
GNAS complex	M21139	1(as)	2	G-protein alpha subunit s, knockout is embryonic lethal	
HSPCA	NM_005348	2(s)	27	Chaperone, protein folding	Overexpressed in breast ca, activates tyrosine kinases
EIF-3	NM_003750	3(s)	3	Translation initiation factor	
RPL31	NM_013403	2(s)	9	Ribosomal protein L31	
RPL35	NM_004632	2(as)	4	Ribosomal protein L35	
RPL12	NM_000976	1(s)	2	Ribosomal protein L12	
RPS29	NM_001032	1(s)	2	Ribosomal protein S29	
RPS24	NM_001026	1(s), 1(as)	8	Ribosomal protein S24	
18S RNA	K03432.1	8(s), 4(as)	33	Ribosomal RNA	
28S RNA	M27830	3 (s)	17	Ribosomal RNA	
7SL	X04248	4(s), 1(as)	8	RNA component of signal recognition particle	

Table 3. Enriched Genes That Have Not Been Previously Implicated in Cell Proliferation

Gene	Accession No.	#Sequences (s/as)	# clones	Description	Association with cancer
Transcription factors					
ATF4	NM_001675	5(as)	369	Activating transcription factor	Induced in breast ca by heregulin
HES6	XM_043579	1(s)	6	Transcription co-factor, differentiation inducer	
NR3C1	NM_000176	1(s)	5	Glucocorticoid receptor	
EDF1	NM_003792	1(s)	2	Transcription factor, stimulates endothelial cell growth, represses endothelial cell differentiation	
MBD1	NM_015847	1(s), 1(as)	2	Methylated DNA binding protein, transcription inhibitor	
RNA transport					
HRPMT1L2	NM_001536	1(s)	5	Hnrp arginine methyltransferase	
HNRPF	NM_004966	1(s)	5	Heterogeneous nuclear ribonucleoprotein F	
HNRPA2B1	NM_002137	1(s)	4	Heterogeneous nuclear ribonucleoprotein A2/B1	
Signal transduction and cell adhesion					
ZIN	NM_013403	1(as)	6	Calmodulin-binding WD repeat protein	
Arfaptin1	NM_014447	1(as)	2	Similar to POR1 GTP-binding protein; may act in cellular membrane ruffling and formation of lamellipodia	
L1CAM	NM_000425	8(s), 4(as)	20	Cell adhesion, neural	
ICAM2	NM_000873	2(s), 1(as)	8	Cell adhesion, intercellular	
Intracellular transport					
AP1B1/BAM22	NM_001127	2(s)	5	Clathrin-associated adaptor protein	
RAB2L	NM_004761	1(s)	4	Small GTPase, intracellular transport	Ras family
KIFC1	NM_042626	1(as)	3	Intracellular trafficking	

Gene	Accession No.	#Sequences (s/as)	# clones	Description	Association with cancer
Rab5B	NM_002868	1(s), 1(as)	3	Small GTPase, vesicle transport	Ras family
Protein processing					
NIN283	NM_032268	1(s)	11	ubiquitin-mediated protein modification	
PSMB7	NM_002799	1(s)	4	Proteasome subunit β7	
SQSTM1	NM_003900	1(s)	2	Sequestosome 1; ubiquitin-mediated protein degradation	
RAD23A	NM_005053	1(s)	2	Nucleotide excision repair, ubiquitin-mediated protein degradation	
Other					
VWF	NM_000552	6(s), 5(as)	39	Blood clotting	
GSTP	NM_000852	2(s)	8	Xenobiotic metabolism	
ENO1	NM_001428	2(s)	8	Glycolysis	
IF1	NM_016311	1(s)	4	Inhibitor of Fo/F1 mitochondrial ATPase	
MYL6	NM_021019	2(s)	2	Contractility	
FLJ13052	NM_023018	1(s)	2	NAD kinase (predicted)	
GBC-14	AL557138	1(s)	2	similar to tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	
KIAA1270	XM_044835	1(as)	9	Alanyl-tRNA synthetase homolog	
Unknown function					
GBC-1	NM_031221	2 (s)	70	Contains helical repeat peptide	
FLJ10006	XM_041928	1(as)	2		
GBC-3	AA443027	1(s)	12	HC 3q29	
GBC-11		1(s)	4	HC 14	
GBC-12		1(s)	3	HC 1	
GBC-13		1(s)	2		

Gene	Accession No.	#Sequences (s/as)	# clones	Description	Association with cancer
GBC-15	BE079876	1(s)	2		
GBC-16		1(s)	2		
GBC-17		1(s)	2		
GBC-18		1(s)	2		

TABLE 4. Nucleotide Sequences of GSEs

Gene/Accession No.	No. of Clones	Orientation	SEQ ID NO	Sequence
18S RNA K03432.1	1	AS	4	1089 gccgctagaggtgaaattccttggaccggcgcaagacggaccagagcgaaagcatttgccaagaatgttttca ttaatcaagaacgaaagtcggaggttcgaagacgatcagataccgtcgtagttccgaccataaacgatgccgacc ggcgatgcggcggcgttattcccatgacccgccgg 1271
	2	AS	5	1413 ccggacacggacaggattgacagattgatagctctttctcgattccgtgggtggtggtgcatggccgttcttag ttggtggagcgatttgtctggttaattccgataacgaacgaga 1529
	6	S	6	177 caaagattaagccatgcatgtctaagtacgcacggccggtacagtgaaactgcgaatggctcattaaatcagtta tggttcctttggtcgct 268
	7	S	7	1414 cggacacggacaggattgacagattgatagctctttctcgattccgtgggtggtggtgcatggccgttc 1482
	4	AS	8	154 ctgccagtagcatatgcttgtctcaaagattaagccatgcatgtctaagtacgcacggccggtac 218
	1	AS	9	199 taagtacgcacggccggtacagtgaaactgcgaatggctcattaaatcagttatggt 255
	2	S	10	570 cggagagggagcctgagaaacggctaccacatccaaggaaggca 613
	3	S	11	177 caaagattaagccatgcatgtctaagtacgcacggccggta 217
	1	S	12	1040 cggaactgaggccatgattaagagggacggccggg 1074
	1	S	13	1433 cagattgatagctctttctcgattccgtgggtggt 1467
	1	S	14	224 aactgcgaatggctcattaaatcagttatggttcctttggtcgct 268
	4	S	15	185 aagccatgcatgtctaagtacgcacggccg 214
28S RNA M27830.1	10	S	16	83 ccctactgatgatgtgttgttgccatggtaatcctgctcagtacgagaggaaccgcaggttcagacatttggt gtatgtgcttggctgaggagccaatggggcgaacgtaccatctgt 200
	4	S	17	1 gaattcaccaagcgttggattgttcacccactaatagggaacgtgagct 49
	3	S	18	136 cgcaggttcagacatttggtgtatgtg 162
7SL RNA X04248.1	3	S	19	29 cccagctactcgggaggctgaggctggaggatcgcttgagtccaggagttctgggctgtagtgcgctatgc cgatcgggtgtccgcactaagttcggcatcaatatgg 136
	1	S	20	70 ccaggagttctgggctgtagtgcgctatgccgatcgggtgtccgcactaagttcggcatcaatatggt 137
	3	S	21	144 ccgggagcgggggaccaccaggttgcctaaggaggggtga 183
	9	AS	22	24 gtagtcccagctactcgggaggctgaggctggaggatcgcttga 67

Gene/Accession No.	No. of Clones	Orientation	SEQ ID NO	Sequence
	3	S	23	153 ggggaccaccaggttgcctaaggagggtga 183
ADPRT NM_001618	9	S	24	2736 gctgtggcacgggtctaggaccaccaactttgctgggatcctgtcccagggtcttcggatagccc cgcctgaagcgcccgtgacag 2821
	1	AS	25	2422 gaccctcccctgagcagactgtaggccacctcgatgtccagcaggttgtcaagcatttcc acctggcctgcacactgtctgc 2504
ARFAPTIN1 NM_014447	2	AS	26	26 ttcacactgaccaaccgccgaggacagtcggaccggcgacctctcaacccagcc 79
ATF4 NM_001675 .1	359	AS	27	833 acaccttcgaattaagcacatcctcgatccagcaaagcaccgcaacatgaccgaaatgagcttcctgagcagcg 909
	6	AS	28	833 gacaccttcgaattaagcacatcctcgattccagcaaagcaccgcaaca 883
	2	AS	29	838 ----ccttagaattaagcacatcctcgattccagcaaagcgccgcaacatgacggaaa 893
	1	AS	30	843 --------gaattaagcactttcctcgagtccagcaaagccccgca---------- 880
	1	AS	31	864 cgctgctcagcaagctctgttcggtcatgttgcggtgctttgctgg 909
IF1 NM_016311 .1	4	S	32	13 ccagcagcaatggcagtgacggcgttggcggcgcggacgtggcttggcgtgtggggc 69
BAG1 NM_004323 .2	3	S	33	434 ccgggacgaggagtcgacccggagcgaggaggtgaccagggaggaaatggcggcagctgggctcaccgtga ctgtcacccacagc 518
	1	S	34	461 ggaggtgaccagggaggaaatggcggcagctgggctcaccgtgactgtcacccacagc 518
AP1B1 NM_001127 .1	5	S	35	275 gccaagagtcagcctgacatggccattatgggccgtcaacaccttttgtgaaggactgtgagga 336
	1	S	36	286 gcctgacatggccattatggccgtcaacaccttttgtgaaggactgtgag 334
CDC20 NM_001255	4	AS	37	1001 gccagggacaccatgctacggccttgacagccccttgatgctgggtgaatgtctgcagaggaa cccagccaccctctccaggagcactgggccacacattgaccaagttatcattaccaccactggccaaatgtcgtc catctgggcccagcgcagcccacacacttcctggctgtggccactcagtgtggccacatggtgttctgct 1209
CDK10	1	S	38	1159 gccccagccacctccgagggccagagcatgcgctgtaaacc 1199

Gene/Accession No.	No. of Clones	Orientation	SEQ ID NO		Sequence
NM_003674	1	S	39	1734	ctaccaggagagccctgggctggaggctgagctgcatcgctcccacatggaggacccaa caggaggccgtggctctgatgctgagcgaagct 1829
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	2	S	52	3508	tgagaaggaacaagccagggaagcgtgaacaatgatgctctgctgggctgccgctcgggcttct gtacaactgacctggttt 3592
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	1	S	131	253 ccaaggaagagctgggtgtgaccgtgtaccagtcgccccact 294
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	1	S	133	729 gacaggaagccgcgcctgctcttcccccaccaactccagcagccacctggtg 779
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	1	AS	135	2889 cccctggatgagggggcaagggcaact 2917
	7	S	136	94 aatatgaaggacaccatgtgatggagc 120
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RAB5B NM_002868.1	2	S	178	834 aacaccaggcagctgttccgactggcctcct 864
	1	AS	179	1345 gggcggaggtggaggtgcagggtcaactgtggctctgta 1383
RAD23A NM_005053	2	S	180	1351 gcctgctcanagaagctggcaggactgggaggcgacagatgggcccctcttggcctctgtcccagctct 1419
RAN NM_006325	6	S	181	750 ggatggtgacctgtgagaatgaagctggagcccagcgtcagaagtctagttttataggcagctgtcc 816
REL NM_002908	2	S	182	1727 tgaatcttgaaaaccccctcatgtaattcagtgttagacccaagagacttgagacagctcccatc agatgtcctcttccagtatgtcagcaggcgccaattccaatactactgcccattgtttcacaatcagatgcattt gagggatctgacttcagttgtgcagataacagcatgataaatg 1906
AHRG NM_001665.1	36	S	183	518 aggagcagagccaggcgcccatcacaccgcagcagggccaggcactcgcgaaacagatcc acgctgtgcgctacctcgaatgctcagccctgcaacaggatggtgtcaaggaagtgttcgccgag gctgtccgggctgtgctc 660
	2	AS	184	377 ccattgccagtccgccgtcctatgagaacgtgcggcacaagtggcatccagaggtgtgccacca ctgccctgatgtgcccatcctgctggtgggcaccaagaaggacctgagagcccagcctgacaccctacggc 511
	1	S	185	518 aggagcagagccaggcgcccatcacaccgcagcagggccaggcact 563
	2	S	186	273 ggcaatggagaaacagatgacgaaaacgttggtctgagggtaggagagtgtacggaggcggtcatact cctcctggcccgcagtgtcccacaggttcaggttcactttgcgc 384
	1	S	187	516 caccatcctgttgcagggctgagcattcgaggtagcgcacagcgtggatctgcttggccagtgcctggc cctgctgcggtgtgatgggcgcctggccctgctccttg 622

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RPA1 NM_002945	2	AS	189	2163 tggagaagcaaaaacctagttacataatttacttcatggtctgcagttagggtcagtgactta cgacataattcctgcttgatgataatgaaattgacagaagcctgaaggctgagtgagtga 2285
RPA3 NM_002947 .1	6	S	190	8 agccgcagtcttggaccataatcatgg 34
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RPL31 NM_013403 .1	9 1	S S	192 193	28 tggcgagaagaaaaagggccgttctgccatcaacgaagtggtaacccgagaat 80 44 ggccgttctgccatcaacgaagtggtaacccgagaat 80
RPL35 NM_004632 .1	2 1	AS AS	194 195	12 ggcggcttgtgcagcaatggccaagatcaaggctcgagatct 53 12 ggcggcttgtgcagcaatggccaagatcaaggc 44
RPS24 NM_001026	4 4	AS s	196 197	351 gccagcaccaacaacattggcctttgcagtccccctgacttttcttcattctgttcttgcgttcct ttcgttgct 421 373 cagaatgaagaaagtcaggggactgcaaaggccaatgttggtgctggcaaaaag 427
RPS29 NM_001032	2	S	198	4 ttacctcgttgcactgctgagagcaagatgggtcaccagcagctgtactggagcca 59
SQSTM1 NM_003900	2	S	199	1278 ggcagcaaaacaagtgacatgaagggagggtccctgtgtgtgtgc 1324
STAT3 NM_003150 .1	11 7	S AS	200 201	2288 gagagcccaggagcatcctgaagctgacccaggtagcgctgccccatacctgaagaccaagttta tctgtgtgacaccaacgacctgcagcaataccattgacctgccgatgtccccccgc 2407 2111 aagacccagatccagtccgtggaaccatacacaaagcagcagctgaacaacatgtcatttgctgaaatcat catgggctataagatcatggatgctaccaatatcctg 2218
	2	S	202	667 ggatgtccggaagagagtgcaggatcttagaacagaaaatgaaagtggtagagaatctcca ggatgactttgatttcaactataaaaccctcaagagtc 764

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	1	AS	204	834 agatgctcactgcgctggaccagatgcggagaagcatcgtgagtggcgggggctttgtcagcgatggagt acgtgcagaa 918
	1	S	205	402 gaccagcagtatagccgcttcctgcaagagtcgaatgttctctatca 459
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STAT5b NM_012448 .1	102	AS	207	287 tcttgataatccacaggagaacattaaggccacccagctcctggagggcctggtgcag gagctgcagaagaaggcagaacaccaggtgggggaagatgggtttttt 391
	1	AS	208	303 gagaacattaaggccacccagctcctggagggcctggcgcaggagctgcagaacaaggcacaacaccagg aggggaagatg 384
	3	S	209	1941aacaagcagcaggcccacgacctgctcatcaacaagccagatgggaccttcctgctgcgcttcagcgactcggaaa tcgggggcatcaccattgcttggaagtttga 2047
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VWF NM_000552 .2	8	S	217	3646 ccagcatggcaaggtggtgacctggaggacggccacattgtgcccccagagctgcgaggagaggaatctccgggga gaacgggtatgagtgtgagtggcgctataacagctgtgcacctgcctg 3768
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	2	S	220	7776 agtgctgtggaaggtgcctgccatctgcctgtgaggtggtgactggctcaccgcgggggggactcccagtcttcct g 7851
	2	S	221	5082 tggtcagccagggtgaccgggagcaggcgcccaacctggtctacatggtcaccggaaatccctg 5144
	3	S	222	6003 agtgccacaccgtgacttgccagccagatggccagaccttgctgaagagtcatcgggtcaactgt 6067
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	1	AS	225	7818 ctggctcaccgcgggggggactcccagtcttcctggaagagtgtcggctcccagtggg 7874
	1	AS	226	1380 accctcccggcacctccctcctctcgagactgcaacacctgcatttgccgaaacagcc 1436
	2	AS	227	8762 agctgcatgggtgcctgctgctgcc 8786
ZIN NM_013403.1	6	AS	228	1782 ctcagtggccttcaccagcaccgagcctgcccacatcgtggcctccttccgctctggcgacaccgtcttgtatga catggaggttggcagtgccctcctcacgctggagtcccggggcagcagcggtccaaccca 1916

TABLE 5. Peptides encoded by sense-oriented GSEs

Gene	GSE SEQ ID NO	Peptide SEQ ID NO	Location in Parent Protein (AA Residues)	Sequence
ADPRT	24	229	860-887	LWHGSRTTNFAGILSQGLRIAPPEAPVT
IF1	32	230	1-16	MAVTALAARTWLGVWG
BAG1	33	231	53-80	RDEESTRSEEVTREEMAAAGLTVTVTHS
	34	232	62-80	EVTREEMAAAGLTVTVTHS
AP1B1	35	233	76-97	YAKSQPDMAIMAVNTFVKDCED
	36	234	81-96	PDMAIMAVNTFVKDCE
CDK10	38	235	347-360	APATSEGQSKRCKP
CDK2	40	236	51-66	EISLLKELNHPNIVKL
	41	237	159-177	YTHEVVTLWYRAPEILLGC
c-FOS	45	238	362-378	PELVHYREEKHVFPQRF
	47	239	148-158	KMAAAKCRNRR
CREB1	54	240	27-49	VQAQPQIATLAQVSMPAAHATSS
CCND1	55	241	56-91	MRKIVATWMLEVCEEQKCEEEVFPLAMN YLDRFLSL
EDF1	61	242	22-37	AKSKQAILAAQRRGGD
EIF1	62	243	1050-1063	RGGADDERSSWRNA
EFNA1	67	244	53-79	HYEDHSVADAAMEQYILYLVEHEEYQL
FEN1	71	245	90-101	PQLKSGELAKRS
FGFR1	76	246	427-470	VTVSADSSASMNSGVLLVRPSRLSSSGTPMLAGVSEYELPED PR
	78	247	402-421	GTKKSDFHSQMAVHKLAKSI

Gene	GSE SEQ ID NO	Peptide SEQ ID NO	Location in Parent Protein (AA Residues)	Sequence
GBC1	79	248	36-54	LTSQTMGGQAETLLTSQKG
FOS2L	83	249	246-261	IKPISIAGGFYGEEPL
GSTP	97	250	83-102	DQQEAALVDMVNDGVEDLRC
	98	251	170-210	CLDAFPLLSAYVGRLSARPKLKAFLASP EYVNLPINGNGKQ
GBC-3	94	252		WMDGRDEVTQQKYQRPETEWPRVSLH PEPEDAAKTSLSE
HES6	99	253	874-948	RAAPGNQPSQAPAPFLKKLLGTLQL
HNRPA2B1	100	254	786 -866	ISDQDQEVTLEEDLMDMAVDVDLGMAI
HNRPF	101	255	226-278	AGLERMRPGAYSTGYGGYEEYSGLSDGYGFTTDLFGRDLSY CLSGMYDHRYGD
HRMT1L2	102	256	2701-2748	GVGAGEDGGSRGRELH
HSPCA	103	257	499-515	KDQVANSAFVERLRKHG
ICAM2	105	258	1-19	MSSFGYRTLTVALFTLICC
	106	259	216-229	YEPVSDSQMVIIVT
IGF2R	108	260	253-265	KLVRKDRLVLSYV
	109	261	481-496	KKRYDLSALVRHAEPE
INTB5	111	262	12-56	LLGLCALLPRLAGLNICTSGSATSCEECLLIHPKCAWCSKEDF GS
	112	263	688-724	KDCVMMFTYVELPSGKSNLTVLREPECGNTPNAMTIL
	113	264	457-485	GHGECHCGECKCHAGYIGDNCNCSTDIST
	114	265	697-726	VELPSGKSNLTVLREPECGNTPNAMTILLA

Gene	GSE SEQ ID NO	Peptide SEQ ID NO	Location in Parent Protein (AA Residues)	Sequence
ITGA4	119	266	18-41	PEAAVRETVMLLLCLGVPTGRPYN
JUNB	121	267	19-34	GYGRAPGGLSLHDYKL
	122	268	24-32	PGGLSLHDY
L1CAM	127	269	457-491	SVQWLDEDGTTVLQDERFFPYANGTLGIRDLQAND
	129	270	216-251	TRTIIQKEPIDLRVKATNSMIDRKPRLLFPTNSSSH
	130	271	191-220	GNLYFANVLTSDNHSDYICHAHFPGTRTII
	132	272	450-469	AFGAPVPSVQWLDEDGTTVL
	134	273	25-39	EYEGHHVMEPPVITE
	131	274	79-91	KEELGVTVYQSPH
	133	275	237-253	DRKPRLLFPTNSSSHLV
PSMB7	139	276	193-211	EAKNLVSEAIAAGIFNDLG
MCM3	145	277	519-543	PLGSAVDILATDDPNFSQEDQQDTQ
	148	278	789-802	LSKMQDDNQVMVSE
MCM6	151	279	690-711	PAPVNGINGYNEDINQESAPKA
MET	154	280	1253-1317	YSVHNKTGAKLPVKWMALESLQTQKFTTKSDVWSFGVVLW ELMTRGAPP YPDVNTFDITVYLLQG
MYL6	155	281	2-23	MCDFTEDQTTEFKEAFQLFDRT
	156	282	7-32	EDQTTEFKEAFQLFDRTGDGKILYNQ
NR3C1	159	283	132-155	STSVPENPKSSASTAVSAAPTEKE
NUMA1	160	284	1314-1332	ELTSQAERAEELGQELKAW
GRP58	161	285	360-382	NLKRYLKSEPIPESNDGPVKVVV
PC4	163	286	32-49	APEKPVKKQKTGETSRAL
PKC delta	165	287	281-322	GINQKLLAEALNQVTQRASRRSDSASSEPVGIYQGFEKKTGV
	166	288	204-239	IIGRCTGTAANSRDTIFQKERFNIDMPHRFKVHNYM

Gene	GSE SEQ ID NO	Peptide SEQ ID NO	Location in Parent Protein (AA Residues)	Sequence
PKCeta	168	289	55-84	GQTSTKQKTNKPTYNEEFCANVTDGGHLEL
	169	290	73-106	CANVTDGGHLELAVFHETPLGYDHFVANCTLQFQE
PKCzeta	172	291	130-148	GHLFQAKRFNRRAYCGQCS
	173	292	55-94	PLTLKWVDSEGDPCTVSSQMELEEAFRLARQCRDEGLIIH
RAB2L	177	293	291-321	VTQFNKVAGAVVSSVLGATSTGEGPGEVTIR
REL	182	294	518-553	NLENPSCNSVLDPRDLRQLHQMSSSSMSAGANSNTT
AHRG	183	295	131-177	EQSQAPITPQQGQALAKQIHAVRYLECSALQQDGVKEVFAEA VRAVL
	186	296	49-85	GWMEEQSQAPITPQQGQALE
	187	297	130-164	KEQSQAPITPQQGQALAKQIHAVRYLECSALQQDG
	188	298	138-155	TPQQGQALAKQIHAVRYL
RPL12	191	299	209-228	SRIRVHLTPAASTMLPKFNP
RPL31	192	300	8-24	GEKKKGRSAINEVVTRE
RPS24	197	301	113-130	WMDGRMKKVRGTAKANVGAGKK
STAT3	200	302	6150 - 729	ESQEHPEADPGSAAPYLKTKFICVTPTTCSNTIDLPMSPR
	202	303	90-181	DVRKRVQDLEQKMKVVENLQDDFDFNYKTLKS
	203	304	71-108	FLQESNVLYQHNLRRIKQFLQSRYLEKPMEIARIVARC
	205	305	65 -79	DQQYSRFLQESNVLY
STAT5	209	306	599-633	NKQQAHDLLINKPDGTFLLRFSDSEIGGITIAWKF
	210	307	422-455	KRIKRSDRRGAESVTEEKFTILFESQFSVGGNEL
	213	308	441-455	TILFESQFSVGGNEL
VWF	217	309	1113-1152	QHGKVVTWRTATLCPQSCEERNLRENGYECEWRYNSCAPA
	219	310	272-299	ARTCAQEGMVLYGWTDHSACSPVCPAGM
	220	311	2490-2513	CCGRCLPSACEVVTGSPRGDSQSS
	221	312	1592-1611	VSQGDREQAPNLVYMVTGNP
	222	313	1899-1919	CHTVTCQPDGQTLLKSHRVNC
	224	314	1356-1376	STSEVLKYTLFQIFSKIDRPE

We claim:

1. A method for identifying a compound that inhibits growth of a mammalian cell, the method comprising the steps of:

(a) culturing a cell in the presence or absence of the compound;

(b) assaying the cell for expression or activity of one or a plurality of the genes set forth in Table 3; and

(c) identifying the compound when expression or activity of at least one of the genes set forth in Table 3 is lower in the presence of the compound than in the absence of the compound.

2. A method according to claim 1, wherein the cell is a tumor cell.

3. A method according to claim 2, wherein the cell is a human tumor cell.

4. A method according to claim 1, further comprising the step of comparing cell growth in the presence of the compound with cell growth in the absence of the compound.

5. The method of claim 1, where expression of the cellular gene of Table 3 is detected by hybridization to a complementary nucleic acid.

6. The method of claim 1, wherein expression of the cellular gene of Table 3 is detected using an immunological reagent.

7. The method of claim 1, wherein expression of the cellular gene of Table 3 is detected by assaying for an activity of the cellular gene product.

8. A method according to claim 1, wherein expression of a cellular gene in Table 3 is assayed using a recombinant mammalian cell comprising a reporter gene operably linked to a promoter from a cellular gene in Table 3 and detecting decreased expression of the reporter gene in the presence of the compound than in the absence of the compound.

9. The method of claim 8, further comprising the step of assaying cell growth in the presence and absence of the compound and identifying compounds that inhibit cell growth and a gene identified in Table 3.

10. A method according to claim 9, wherein the cell is a tumor cell.

11. A method according to claim 10, wherein the cell is a human tumor cell.

12. The method of claim 9, where expression of the cellular gene of Table 3 is detected by hybridization to a complementary nucleic acid.

13. The method of claim 9, wherein expression of the cellular gene of Table 3 is detected using an immunological reagent.

14. The method of claim 9, wherein expression of the cellular gene of Table 3 is detected by assaying for an activity of the cellular gene product.

15. A compound that inhibits tumor cell growth identified according to a method of claim 1, 4, 8 or 9 and wherein the compound is not an inhibitor of RNA synthesis or protein synthesis.

16. A target gene for identifying compounds that inhibit tumor cell growth, wherein the target gene is a gene identified in Table 3 and inhibition of expression of the gene or activity of the gene product inhibits growth of a tumor cell.

17. A method for inhibiting tumor cell growth, the method comprising the steps of contacting a tumor cell with an effective amount of a compound that inhibits expression of a gene in Table 3.

18. A method for inhibiting tumor cell growth, the method comprising the steps of contacting a tumor cell with an effective amount of a compound that

inhibits expression of a gene in Table 3, wherein the compound is identified according to the method of claims 1, 4, 8 or 9.

19. A method for assessing efficacy of a treatment of a disease or condition relating to abnormal cell proliferation or tumor cell growth, the method comprising the steps of:

(a) obtaining a biological sample comprising cells from an animal having a disease or condition relating to abnormal cell proliferation or tumor cell growth before treatment and after treatment with a compound according to claim 15;

(b) comparing expression of at least one gene in Table 3 after treatment with a compound according to claim 15 with expression of said genes before treatment with a compound according to claim 15; and

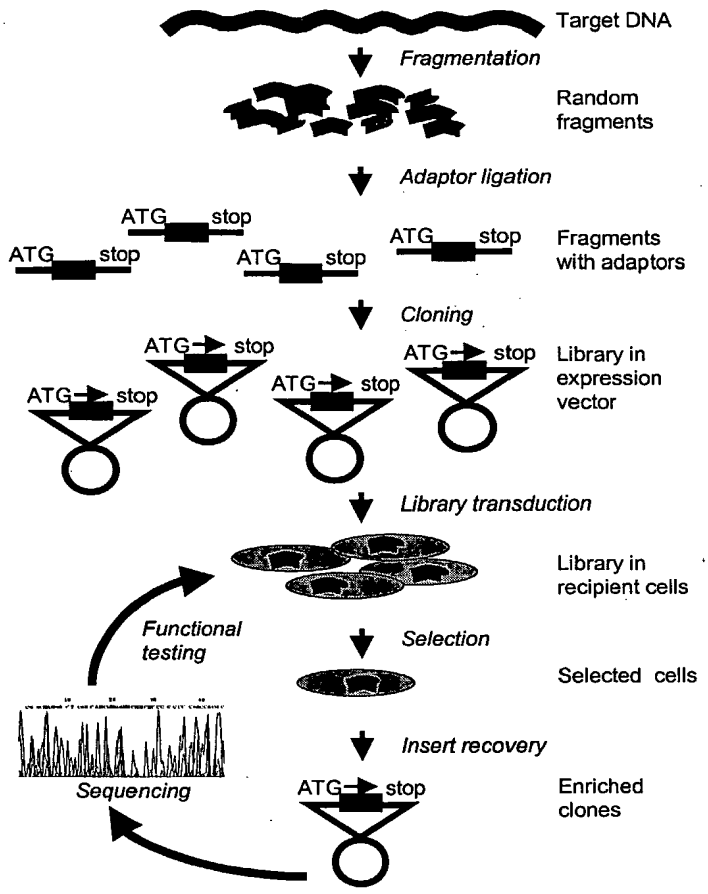
(c) determining that said treatment with a compound according to claim 15 has efficacy for treating the disease or condition relating to abnormal cell proliferation or tumor cell growth if expression of at least one gene in Table 3 is lower after treatment than before treatment.

20. A method for treating a disease or condition relating to abnormal cell proliferation or tumor cell growth, the method comprising the steps of administering to an animal having said disease or condition a therapeutically effective amount of a compound produced according to the method of claim 1, 4, 8 or 9 that inhibits expression of a gene in Table 3.

21. A compound that inhibits in a mammalian cell expression or activity of a gene identified in Table 3 wherein the compound is identified according to a method of claim 1, 4, 8 or 9 and wherein the compound is not an inhibitor of RNA synthesis or protein synthesis.

22. A peptide having an amino acid sequence of any one of the sequences identified by SEQ ID NOS. 229-314.

23. A peptidomimetic, organomimetic or chemical mimetic of a peptide according to claim 22, wherein said mimetic has substantially the same tumor cell growth inhibiting activity as the peptide for which it is a mimetic.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Random fragments
Adaptor ligation
ATG stop
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Cloning
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Functional testing
Selection
Selected cells
Insert recovery
Sequencing
Enriched clones

Figure 1

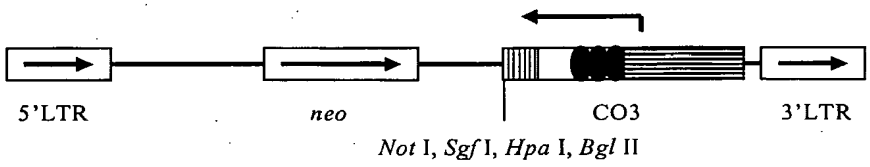


Figure 2

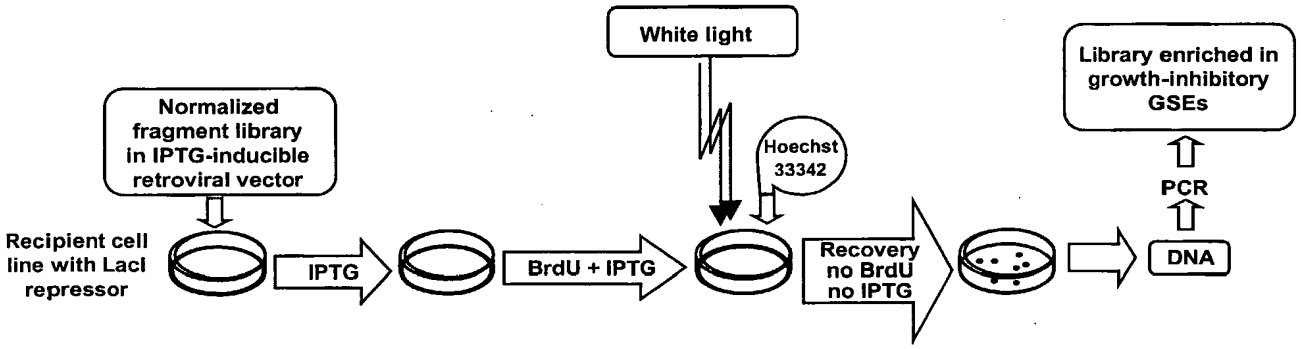

White light
Library enriched in growth-inhibitory GSEs
Normalized fragment library in IPTG-inducible retroviral vector
Hoechst 33342
PCR
Recipient cell line with LacI repressor
IPTG
BrdU + IPTG
Recovery no BrdU no IPTG
DNA

Figure 3

Library-transduced cells -IPTG +IPTG

G418-selected

One round BrdU

Two rounds BrdU

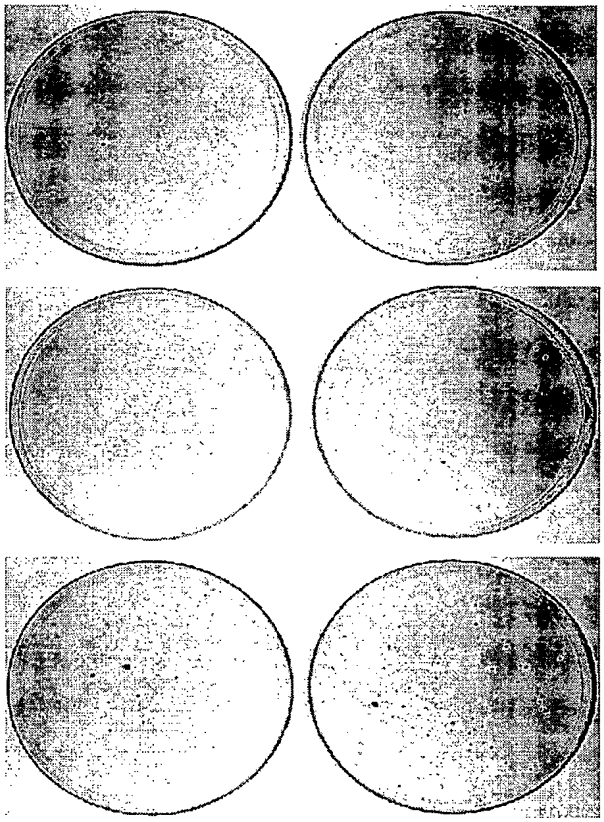
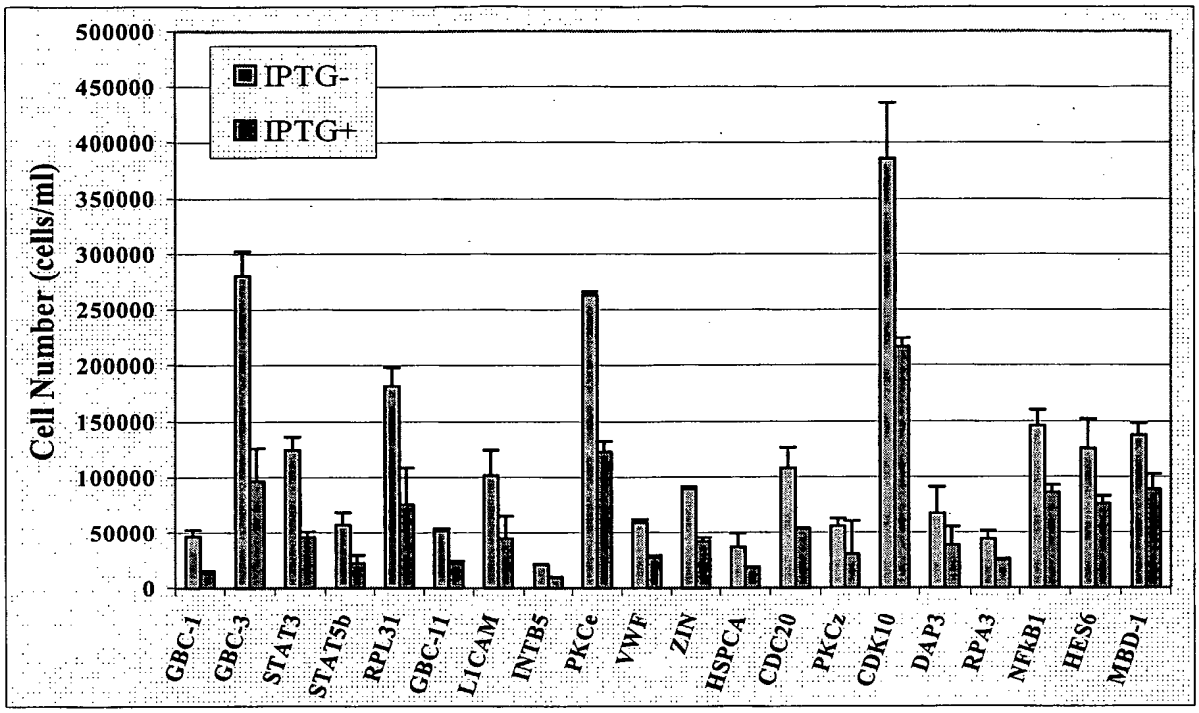


Figure 4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IPTG+
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150000
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50000
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GBC-3
STAT3
STAT5b
RPL31
GBC-11
L1CAM
INTB5
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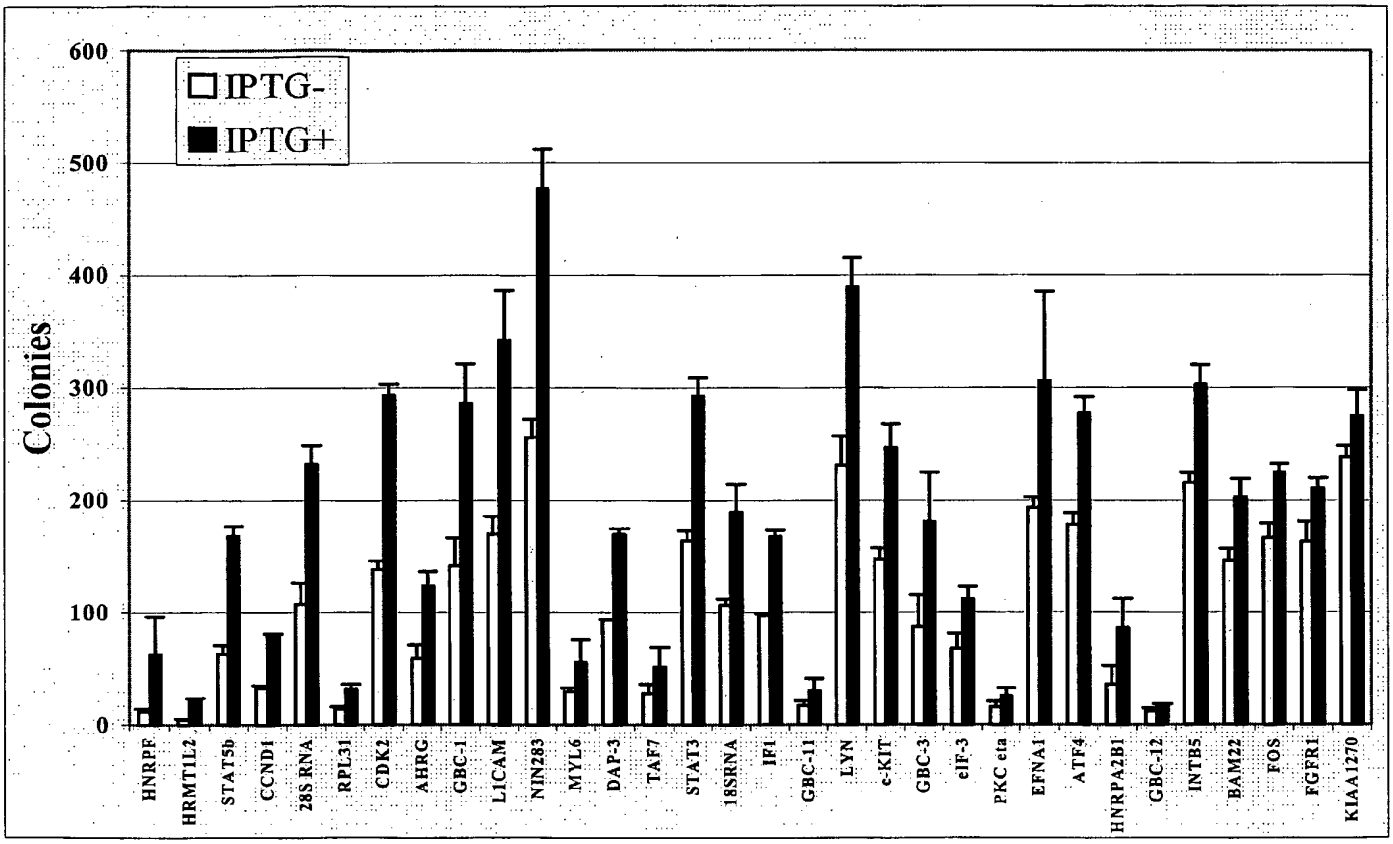
MBD-1

Figure 5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NIN283
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STAT3
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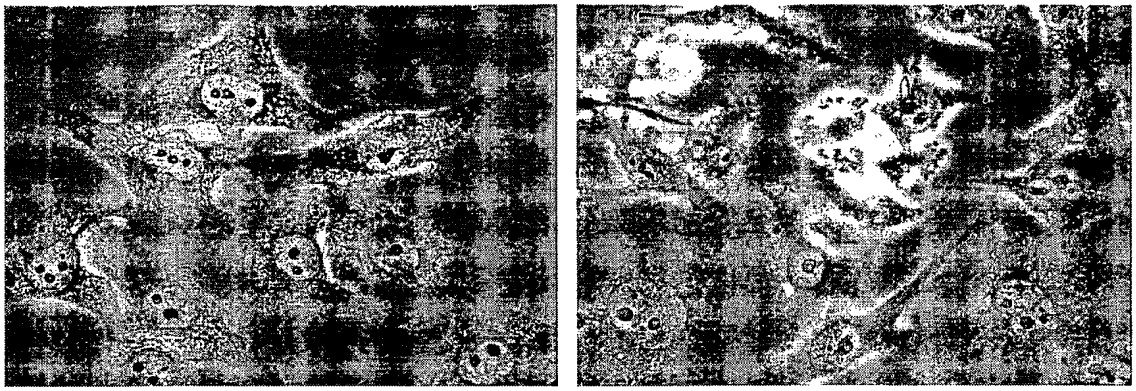
FOS
FGFR1
KIAA1270

Figure 6

A. Cell morphology



no IPTG 4 days IPTG

B. Mitotic catastrophe in IPTG-treated cells

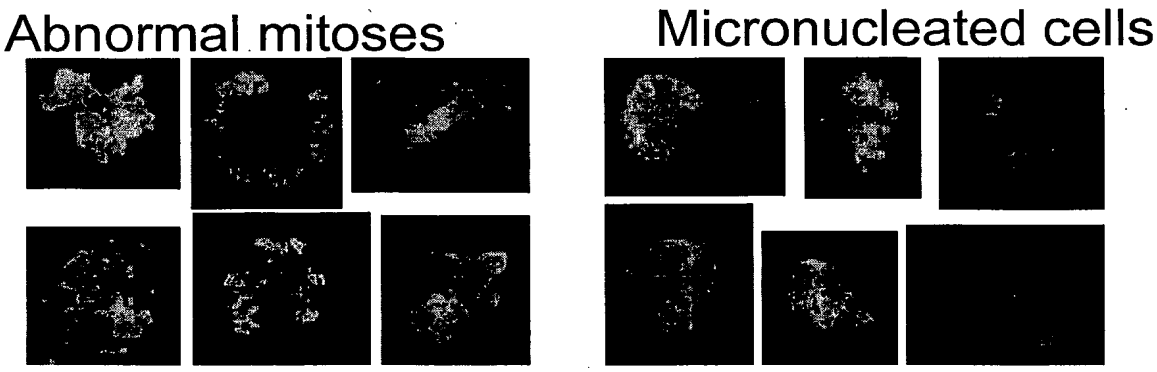


Figure 7

SEQUENCE LISTING

<110> Board of Trustees of the University of Illinois
Primiano, Thomas
Chang, Bey-dih
Roninson, Igor

<120> Methods and Reagents for Identifying Gene Targets for Treating Cancer

<130> 99,216-U

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cagtgagggt tggg 134

<210> 99
<211> 79
<212> DNA
<213> Homo Sapiens

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tagggaccct gcagctctg 79

<210> 100
<211> 77
<212> DNA
<213> Homo Sapiens

<400> 100
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atttggggat ggctata 77

<210> 101
<211> 158
<212> DNA
<213> Homo Sapiens

<400> 101

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acagtggcct cagtgatggc tacggcttca ccaccgacct gttcgggaga gacctcagct 120

actgtctctc cggaatgtat gaccacagat acgccgac 158

<210> 102
<211> 42
<212> DNA
<213> Homo Sapiens

<400> 102
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<210> 103
<211> 52
<212> DNA
<213> Homo Sapiens

<400> 103
caaggaccag gtagctaact cagcctttgt ggaacgtctt cggaaacatg gc 52

<210> 104
<211> 36
<212> DNA
<213> Homo Sapiens

<400> 104
ccaaggacca ggtagctaac tcagcctttg tggaac 36

<210> 105
<211> 101
<212> DNA
<213> Homo Sapiens

<400> 105
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tacaggaccc tgactgtggc cctcttcacc ctgatctgct g 101

<210> 106
<211> 64
<212> DNA
<213> Homo Sapiens

<400> 106
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ctgt 64

<210> 107

<211> 106
<212> DNA
<213> Homo Sapiens

<400> 107
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cagagcagga cagatgtcac gaacagggac agcaacaccg acacca 106

<210> 108
<211> 39
<212> DNA
<213> Homo Sapiens

<400> 108
gaagctggtg cgcaaggaca ggcttgtcct gagttacgt 39

<210> 109
<211> 62
<212> DNA
<213> Homo Sapiens

<220>
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<222> (18)..(18)
<223> n stands for a, c, t, or g

<400> 109
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cc 62

<210> 110
<211> 124
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<222> (26)..(26)
<223> n stands for a, c, t, or g

<400> 110
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tgctctctgg ccagaggact gtcttgctga tctccactgg gcaccatgct gattttccag 120

agcc 124

<210> 111
<211> 132

<212> DNA
<213> Homo Sapiens

<400> 111
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ccacctcatg tgaagaatgt ctgctaatcc acccaaaatg tgcctggtgc tccaaagagg 120

acttcggaag cc 132

<210> 112
<211> 115
<212> DNA
<213> Homo Sapiens

<400> 112
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ccgtcctcag ggagccagag tgtggaaaca cccccaacgc catgaccatc ctcct 115

<210> 113
<211> 87
<212> DNA
<213> Homo Sapiens

<400> 113
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tgtaactgct cgacagacat cagcaca 87

<210> 114
<211> 91
<212> DNA
<213> Homo Sapiens

<400> 114
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acccccaacg ccatgaccat cctcctggct g 91

<210> 115
<211> 36
<212> DNA
<213> Homo Sapiens

<400> 115
tgaaagatga ccaggaggct gtgctatgtt tctaca 36

<210> 116
<211> 90
<212> DNA
<213> Homo Sapiens

<400> 116
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ctggctgctt gggggaccaa gaacttgcat 90

<210> 117
<211> 81
<212> DNA
<213> Homo Sapiens

<400> 117
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agatgtatgt ggagttacat g 81

<210> 118
<211> 93
<212> DNA
<213> Homo Sapiens

<400> 118
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aatttccgga cgtctctgct gtactggagc ctg 93

<210> 119
<211> 90
<212> DNA
<213> Homo Sapiens

<400> 119
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gggggtcccg accggccgcc cctacaacgt 90

<210> 120
<211> 53
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<222> (44)..(44)
<223> n stands for a, c, t, or g

<400> 120
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<210> 121
<211> 48

<212> DNA
<213> Homo Sapiens

<400> 121
cgggatacgg ccgggcccct ggtggcctct ctctacacga ctacaaac 48

<210> 122
<211> 28
<212> DNA
<213> Homo Sapiens

<400> 122
ccctggtggc ctctctctac acgactac 28

<210> 123
<211> 96
<212> DNA
<213> Homo Sapiens

<400> 123
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ctctgtatac agttgcaaca cctgggcctc acaggt 96

<210> 124
<211> 69
<212> DNA
<213> Homo Sapiens

<400> 124
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tggaggcaa 69

<210> 125
<211> 93
<212> DNA
<213> Homo Sapiens

<400> 125
ttggggaccc aggagacgac acttggatgt tgtgtggtgg gtaccgaagg cagcgtgtgt 60

atggagctcc tgaaagccgg ccatggggtg ggc 93

<210> 126
<211> 112
<212> DNA
<213> Homo Sapiens

<400> 126
caggcaatcc ctgagctgga aggcattgaa atcctcaact caagtgccgt gctggtcaag 60

tggcggccgg tggacctggc ccaggtcaag ggccacctcc gcggatacaa tg 112

<210> 127
<211> 107
<212> DNA
<213> Homo Sapiens

<400> 127
agtgttcagt ggctggacga ggatgggaca acagtgcttc aggacgaacg cttcttcccc 60

tatgccaatg ggaccctggg cattcgagac ctccaggcca atgacac 107

<210> 128
<211> 106
<212> DNA
<213> Homo Sapiens

<400> 128
gccaatgacc aaaacaatgt taccatcatg gctaacctga aggttaaaga tgcaactcag 60

atcactcagg ggccccgcag cacaatcgag aagaaaggtt ccaggg 106

<210> 129
<211> 109
<212> DNA
<213> Homo Sapiens

<400> 129
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attgacagga agccgcgcct gctcttcccc accaactcca gcagccacc 109

<210> 130
<211> 90
<212> DNA
<213> Homo Sapiens

<400> 130
ggcaacctct actttgccaa tgtgctcacc tccgacaacc actcagacta catctgccac 60

gcccacttcc caggcaccag gaccatcatt 90

<210> 131
<211> 42
<212> DNA
<213> Homo Sapiens

<400> 131
ccaaggaaga gctgggtgtg accgtgtacc agtcgcccca ct 42

<210> 132

<211> 61
<212> DNA
<213> Homo Sapiens

<400> 132
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t 61

<210> 133
<211> 51
<212> DNA
<213> Homo Sapiens

<400> 133
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<210> 134
<211> 40
<212> DNA
<213> Homo Sapiens

<400> 134
aatatgaagg acaccatgtg atggagccac ctgtcatcac 40

<210> 135
<211> 29
<212> DNA
<213> Homo Sapiens

<400> 135
cccctggatg agggggggcaa ggggcaact 29

<210> 136
<211> 27
<212> DNA
<213> Homo Sapiens

<400> 136
aatatgaagg acaccatgtg atggagc 27

<210> 137
<211> 111
<212> DNA
<213> Homo Sapiens

<400> 137
tacatcatca ccgagttcat ggctaagggt agtttgctgg atttcctcaa gagtgatgaa 60

ggtggcaagg tgctgctgcc caagctcatt gacttctcgg cccagattgc a 111

<210> 138
<211> 48
<212> DNA
<213> Homo Sapiens

<400> 138
ggctgtacgc tgtggtcacc aaggaggagc ccatctacat catcaccg 48

<210> 139
<211> 52
<212> DNA
<213> Homo Sapiens

<400> 139
caagaatctg gtgagcgaag ccatcgcagc tggcatcttc aacgacctgg gc 52

<210> 140
<211> 41
<212> DNA
<213> Homo Sapiens

<400> 140
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<210> 141
<211> 97
<212> DNA
<213> Homo Sapiens

<400> 141
ccgctccgga gccatgtagg agcgcgtgcc cacgaaggag ttggccatgg agtctatgag 60

ctggccgctc accccgaagt cacacagctt gatctcc 97

<210> 142
<211> 61
<212> DNA
<213> Homo Sapiens

<400> 142
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g 61

<210> 143
<211> 40
<212> DNA
<213> Homo Sapiens

<400> 143
tttaatacga ctcactatag ggaatttggc cctcgaggcc 40

<210> 144
<211> 188
<212> DNA
<213> Homo Sapiens

<400> 144
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atccaggttg aaggcattca aggtggccct cttggatgtg ttccgggaag ctcatgcgca 120

gtcaatcggc atgaatcgcc tcacagaatc catcaaccgg gacagcgaag agcccttctc 180

ttcagttg 188

<210> 145
<211> 79
<212> DNA
<213> Homo Sapiens

<400> 145
tgcccttggg tagtgctgtg gatatcctgg ccacagatga tcccaacttt agccaggaag 60

atcagcagga cacccagat 79

<210> 146
<211> 77
<212> DNA
<213> Homo Sapiens

<400> 146
accaagaaga aaaaggagaa gatggtgagt gcagcattca tgaagaagta catccatgtg 60

gccaaaatca tcaagcc 77

<210> 147
<211> 79
<212> DNA
<213> Homo Sapiens

<400> 147
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atcagcagga cacccagat 79

<210> 148
<211> 41
<212> DNA
<213> Homo Sapiens

<400> 148
tgagcaagat gcaggatgac aatcaggtca tggtgtctga g 41

<210> 149
<211> 97
<212> DNA
<213> Homo Sapiens

<400> 149
acccaagttc ggagacgagg cctcctcaga tgaggaagat gatgccctca gacaccatga 60

cctgattgtc atcctgcatc ttgctcagag caacctg 97

<210> 150
<211> 80
<212> DNA
<213> Homo Sapiens

<400> 150
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acttcgtgag tacttttggg 80

<210> 151
<211> 68
<212> DNA
<213> Homo Sapiens

<400> 151
gccctgctcc tgtgaacggg atcaatggct acaatgaaga cataaatcaa gagtctgctc 60

ccaaagcc 68

<210> 152
<211> 46
<212> DNA
<213> Homo Sapiens

<400> 152
tggagtgcat tctgacggaa aaagaagaaa gagatccaca agggaa 46

<210> 153
<211> 90
<212> DNA
<213> Homo Sapiens

<400> 153
gcacagtgaa ttttctttcc gtcactgtcc aacaaagtcc catgatagac acagccaaaa 60

tgccctcttc ctatgacttc attgaaatgc 90

<210> 154
<211> 196
<212> DNA

<213> Homo Sapiens

<400> 154
tatagcgtcc acaacaaaac gggtgcgaaa ctaccggtga agtggatggc tttagagagt 60

ctgcagacgc aaaagttcac caccaagtca gacgtgtggt ccttcggtgt gcttctctgg 120

gagctcatga cgagaggagc ccctccttat cctgacgtga acacatttga tatcactata 180

tacctgttgc aaggca 196

<210> 155
<211> 73
<212> DNA
<213> Homo Sapiens

<400> 155
gtcaagatgt gtgacttcac cgaagaccag accgcagagt tcaaggaggc cttccagctg 60

tttgaccgaa cag 73

<210> 156
<211> 82
<212> DNA
<213> Homo Sapiens

<400> 156
ccgaagacca gaccgcagag ttcaaggagg ccttccagct gtttgaccga acaggtgatg 60

gcaagatcct gtacagccag tg 82

<210> 157
<211> 46
<212> DNA
<213> Homo Sapiens

<400> 157
ggccaccgga gcggcccggc gacgatcgct gacagcttcc cctgcc 46

<210> 158
<211> 86
<212> DNA
<213> Homo Sapiens

<400> 158
ggcacccctt ctgcactgac ttccagatat ggttctccct tcctccctga ggacaccaaa 60

ttggatgaga gcaagtttga gagaag 86

<210> 159
<211> 88
<212> DNA

<213> Homo Sapiens

<400> 159
gcaaacctca tatgtcgacc agtgttccag agaaccccaa gagttcagca tccactgctg 60

tgtctgctgc ccccacagag aaggagtt 88

<210> 160
<211> 59
<212> DNA
<213> Homo Sapiens

<400> 160
ggagctgacc tcacaggctg agcgtgcgga ggagctgggc caagaattga aggcgtggc 59

<210> 161
<211> 72
<212> DNA
<213> Homo Sapiens

<400> 161
caatctgaag agatacctga agtctgaacc tatcccagag agcaatgatg ggcctgtgaa 60

ggtagtggta gc 72

<210> 162
<211> 66
<212> DNA
<213> Homo Sapiens

<400> 162
ttagcagttc tgatagcaac aacaggaatc tctccagcag tgctctccaa gtgagtgagc 60

ggccgc 66

<210> 163
<211> 55
<212> DNA
<213> Homo Sapiens

<400> 163
tgctccagaa aaacctgtaa agagacaaaa gacaggtgag acttcgagag ccctg 55

<210> 164
<211> 97
<212> DNA
<213> Homo Sapiens

<400> 164
ccgctacagg caggcgggaa ggaggaaagt ctagctggtt tcggcttcag gagcctcaga 60

gcgagcgggc gaacgtcgcg acgacgggct gagacct 97

<210> 165
<211> 128
<212> DNA
<213> Homo Sapiens

<400> 165
gcggcatcaa ccagaagctt ttggctgagg ccttgaacca agtcacccag agagcctccc 60

ggagatcaga ctcagcctcc tcagagcctg ttgggatata tcagggtttc gagaagaaga 120

ccggagtt 128

<210> 166
<211> 109
<212> DNA
<213> Homo Sapiens

<400> 166
gatcatcggc agatgcactg gcaccgcggc caacagccgg gacactatat tccagaaaga 60

acgcttcaac atcgacatgc cgcaccgctt caaggttcac aactacatg 109

<210> 167
<211> 66
<212> DNA
<213> Homo Sapiens

<400> 167
cacccagaga ctacagtaac tttgaccagg agttcctgaa cgagaaggcg cgcctctcct 60

acagcg 66

<210> 168
<211> 92
<212> DNA
<213> Homo Sapiens

<400> 168
tgggccagac cagcaccaag cagaagacca acaaacccac gtacaacgag gagttttgcg 60

ctaacgtcac cgacggcggc cacctcgagt tg 92

<210> 169
<211> 107
<212> DNA
<213> Homo Sapiens

<400> 169
tgcgctaacg tcaccgacgg cggccacctc gagttggccg tcttccacga gacccccctg 60

ggctacgacc acttcgtggc caactgcacc ctgcagttcc aggagct 107

<210> 170
<211> 75
<212> DNA
<213> Homo Sapiens

<400> 170
aacgaggagt tttgcgctaa cgtcaccgac ggcggccacc tcgagttggc cgtcttccac 60

gagacccccc tgggc 75

<210> 171
<211> 29
<212> DNA
<213> Homo Sapiens

<400> 171
cccacgtaca acgaggagtt ttgcgctaa 29

<210> 172
<211> 60
<212> DNA
<213> Homo Sapiens

<400> 172
acggccacct cttccaagcc aagcgcttta acaggagagc gtactgcggt cagtgcagcg 60

<210> 173
<211> 121
<212> DNA
<213> Homo Sapiens

<400> 173
ccgctcaccc tcaagtgggt ggacagcgaa ggtgaccctt gcacggtgtc ctcccagatg 60

gagctggaag aggctttccg cctggcccgt cagtgcaggg atgaaggcct catcattcat 120

g 121

<210> 174
<211> 158
<212> DNA
<213> Homo Sapiens

<400> 174
gacgtactca atgaccagga acaaccgact tgtcgtctgg aagcaggagt gtaatccgac 60

caggaagggg ttgctggatg cctgctcaaa cacgtgcttc tctgtctgta cccagtcaat 120

atcctcgcca tcatgcacca gctctttctt caccactt 158

<210> 175
<211> 75
<212> DNA
<213> Homo Sapiens

<400> 175
acggaattgc tgtctgattt ctgctttaac agcatttgat gccctgggat agcaaacgct 60

gaacaaacca catgc 75

<210> 176
<211> 89
<212> DNA
<213> Homo Sapiens

<400> 176
aggacccatg gctttctgga gctctgaaaa tctgtcagcc accatatagc gaacgcgcca 60

agatttatct tctgctgctt gtcgaagtg 89

<210> 177
<211> 95
<212> DNA
<213> Homo Sapiens

<400> 177
gtcacacagt ttaacaaggt ggcaggggca gtggttagtt ctgtcctggg ggctacttcc 60

actggagagg gacctgggga ggtgaccata cggcc 95

<210> 178
<211> 31
<212> DNA
<213> Homo Sapiens

<400> 178
aacaccaggc agctgttccg actggcctcc t 31

<210> 179
<211> 39
<212> DNA
<213> Homo Sapiens

<400> 179
gggcggaggt ggaggtgcag ggtcaactgt ggctctgta 39

<210> 180
<211> 69
<212> DNA
<213> Homo Sapiens

<220>

<221> misc_feature
<222> (10)..(10)
<223> n stands for a, c, t, or g

<400> 180
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cccagctct 69

<210> 181
<211> 67
<212> DNA
<213> Homo Sapiens

<400> 181
ggatggtgac ctgtgagaat gaagctggag cccagcgtca gaagtctagt tttataggca 60

gctgtcc 67

<210> 182
<211> 181
<212> DNA
<213> Homo Sapiens

<400> 182
tgaatcttga aaacccctca tgtaattcag tgttagaccc aagagacttg agacagctcc 60

atcagatgtc ctcttccagt atgtcagcag gcgccaattc caatactact gcccattgtt 120

tcacaatcag atgcatttga gggatctgac ttcagttgtg cagataacag catgataaat 180

g 181

<210> 183
<211> 143
<212> DNA
<213> Homo Sapiens

<400> 183
aggagcagag ccaggcgccc atcacaccgc agcagggcca ggcactcgcg aaacagatcc 60

acgctgtgcg ctacctcgaa tgctcagccc tgcaacagga tggtgtcaag gaagtgttcg 120

ccgaggctgt ccgggctgtg ctc 143

<210> 184
<211> 135
<212> DNA
<213> Homo Sapiens

<400> 184
ccattgccag tccgccgtcc tatgagaacg tgcggcacaa gtggcatcca gaggtgtgcc 60

accactgccc tgatgtgccc atcctgctgg tgggcaccaa gaaggacctg agagcccagc 120

ctgacaccct acggc 135

<210> 185
<211> 46
<212> DNA
<213> Homo Sapiens

<400> 185
aggagcagag ccaggcgccc atcacaccgc agcagggcca ggcact 46

<210> 186
<211> 112
<212> DNA
<213> Homo Sapiens

<400> 186
ggcaatggag aaacagatga cgaaaacgtt ggtctgaggg taggagagtg tacggaggcg 60

gtcatactcc tcctggcccg cagtgtccca caggttcagg ttcactttgc gc 112

<210> 187
<211> 107
<212> DNA
<213> Homo Sapiens

<400> 187
caccatcctg ttgcagggct gagcattcga ggtagcgcac agcgtggatc tgcttggcca 60

gtgcctggcc ctgctgcggt gtgatgggcg cctggccctg ctccttg 107

<210> 188
<211> 119
<212> DNA
<213> Homo Sapiens

<400> 188
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cgtcctaggt agcgcgcagc gtggatatgc tcggccagtg catggccctg atgcggtgt 119

<210> 189
<211> 123
<212> DNA
<213> Homo Sapiens

<400> 189
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ttacgacata attcctgctt gatgataatg aaattgacag aagcctgaag gctgagtgag 120

tga 123

<210> 190
<211> 27
<212> DNA
<213> Homo Sapiens

<400> 190
agccgcagtc ttggaccata atcatgg 27

<210> 191
<211> 91
<212> DNA
<213> Homo Sapiens

<400> 191
ggccaaggtg caacttcctt cggtcgtccc gaatccgggt tcatccgaca ccagccgcct 60

ccaccatgcc gccgaagttc gaccccaacg a 91

<210> 192
<211> 53
<212> DNA
<213> Homo Sapiens

<400> 192
tggcgagaag aaaaagggcc gttctgccat caacgaagtg gtaacccgag aat 53

<210> 193
<211> 37
<212> DNA
<213> Homo Sapiens

<400> 193
ggccgttctg ccatcaacga agtggtaacc cgagaat 37

<210> 194
<211> 42
<212> DNA
<213> Homo Sapiens

<400> 194
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<400> 195

ggcggcttgt gcagcaatgg ccaagatcaa ggc 33

<210> 196
<211> 71
<212> DNA
<213> Homo Sapiens

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gccagcacca acattggcct ttgcagtccc cctgactttc ttcattctgt tcttgcgttc 60

ctttcgttgc t 71

<210> 197
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<212> DNA
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cagaatgaag aaagtcaggg ggactgcaaa ggccaatgtt ggtgctggca aaaag 55

<210> 198
<211> 56
<212> DNA
<213> Homo Sapiens

<400> 198
ttacctcgtt gcactgctga gagcaagatg ggtcaccagc agctgtactg gagcca 56

<210> 199
<211> 47
<212> DNA
<213> Homo Sapiens

<400> 199
ggcagcaaaa caagtgacat gaagggaggg tccctgtgtg tgtgtgc 47

<210> 200
<211> 120
<212> DNA
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gagagccagg agcatcctga agctgaccca ggtagcgctg ccccatacct gaagaccaag 60

tttatctgtg tgacaccaac gacctgcagc aataccattg acctgccgat gtccccccgc 120

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<212> DNA
<213> Homo Sapiens

<400> 201
aagacccaga tccagtccgt ggaaccatac acaaagcagc agctgaacaa catgtcattt 60

gctgaaatca tcatgggcta taagatcatg gatgctacca atatcctg 108

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<211> 98
<212> DNA
<213> Homo Sapiens

<400> 202
ggatgtccgg aagagagtgc aggatctaga acagaaaatg aaagtggtag agaatctcca 60

ggatgacttt gatttcaact ataaaaccct caagagtc 98

<210> 203
<211> 115
<212> DNA
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<400> 203
ttcctgcaag agtcgaatgt tctctatcag cacaatctac gaagaatcaa gcagtttctt 60

cagagcaggt atcttgagaa gccaatggag attgcccgga ttgtggcccg gtgcc 115

<210> 204
<211> 85
<212> DNA
<213> Homo Sapiens

<400> 204
agatgctcac tgcgctggac cagatgcgga gaagcatcgt gagtgagctg gcggggcttt 60

tgtcagcgat ggagtacgtg cagaa 85

<210> 205
<211> 47
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gaccagcagt atagccgctt cctgcaagag tcgaatgttc tctatca 47

<210> 206
<211> 47
<212> DNA
<213> Homo Sapiens

<400> 206
gagctggctg actggaagag gcggcaacag atggagtacg tgcagaa 47

<210> 207
<211> 105
<212> DNA
<213> Homo Sapiens

<400> 207
tcttgataat ccacaggaga acattaaggc cacccagctc ctggagggcc tggtgcagga 60

gctgcagaag aaggcagaac accaggtggg ggaagatggg ttttt 105

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<211> 82
<212> DNA
<213> Homo Sapiens

<400> 208
gagaacatta aggccaccca gctcctggag ggcctggcgc aggagctgca gaacaaggca 60

caacaccagg agggggaaga tg 82

<210> 209
<211> 107
<212> DNA
<213> Homo Sapiens

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ttcagcgact cggaaatcgg gggcatcacc attgcttgga agtttga 107

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<212> DNA
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<400> 210
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atcttgtttg actcacagtt cagtgttggt ggaaatgagc tggt 104

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<212> DNA
<213> Homo Sapiens

<400> 211
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gctgcagaag aagagcagaa ctccaggtgg gggaagatgg gttt 104

<210> 212
<211> 48

<212> DNA
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<400> 212
tcttgataat ccacaggaga acattaaggc cacccagctc ctggaggg 48

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<211> 47
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<213> Homo Sapiens

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acaatcctgt ttgaatccca gttcagtgtt ggtggaaatg agctggt 47

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<212> DNA
<213> Homo Sapiens

<400> 214
ccagttcagt gttggtggaa atgagctggt 30

<210> 215
<211> 63
<212> DNA
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cgagctgcgc ctctcggcaa gatttcgcgc tgaccatccc gggccctttc atcactaatc 60

ggt 63

<210> 216
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<212> DNA
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<400> 216
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<210> 217
<211> 123
<212> DNA
<213> Homo Sapiens

<400> 217
ccagcatggc aaggtggtga cctggaggac ggccacattg tgcccccaga gctgcgagga 60

gaggaatctc cgggagaacg ggtatgagtg tgagtggcgc tataacagct gtgcacctgc 120

ctg 123

<210> 218
<211> 127
<212> DNA
<213> Homo Sapiens

<400> 218
ccttgcccct gaagcccctc ctcctactct gccccccccac atggcacaag tcactgtggg 60

cccggggctc ttgggggttt cgaccctggg gcccaagagg aactccatgg ttctggatgt 120

ggcgttc 127

<210> 219
<211> 84
<212> DNA
<213> Homo Sapiens

<400> 219
gcccggacct gtgcccagga gggaatggtg ctgtacggct ggaccgacca cagcgcgtgc 60

agcccagtgt gccctgctgg tatg 84

<210> 220
<211> 76
<212> DNA
<213> Homo Sapiens

<400> 220
agtgctgtgg aaggtgcctg ccatctgcct gtgaggtggt gactggctca ccgcgggggg 60

actcccagtc ttcctg 76

<210> 221
<211> 63
<212> DNA
<213> Homo Sapiens

<400> 221
tggtcagcca gggtgaccgg gagcaggcgc ccaacctggt ctacatggtc accggaaatc 60

ctg 63

<210> 222
<211> 65
<212> DNA
<213> Homo Sapiens

<400> 222
agtgccacac cgtgacttgc cagccagatg gccagacctt gctgaagagt catcgggtca 60

actgt 65

<210> 223
<211> 81
<212> DNA
<213> Homo Sapiens

<400> 223
acatggcaca agtcactgtg ggcccggggc tcttgggggt ttcgaccctg gggcccaaga 60

ggaactccat ggttctggat g 81

<210> 224
<211> 65
<212> DNA
<213> Homo Sapiens

<400> 224
tccaccagcg aggtcttgaa atacacactg ttccaaatct tcagcaagat cgaccgccct 60

gaagc 65

<210> 225
<211> 57
<212> DNA
<213> Homo Sapiens

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<210> 226
<211> 57
<212> DNA
<213> Homo Sapiens

<400> 226
accctcccgg cacctccctc tctcgagact gcaacacctg catttgccga aacagcc 57

<210> 227
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<212> DNA
<213> Homo Sapiens

<400> 227
agctgcatgg gtgcctgctg ctgcc 25

<210> 228
<211> 135
<212> DNA
<213> Homo Sapiens

<400> 228
ctcagtggcc ttcaccagca ccgagcctgc ccacatcgtg gcctccttcc gctctggcga 60

caccgtcttg tatgacatgg aggttggcag tgccctcctc acgctggagt cccggggcag 120

cagcggtcca accca 135

<210> 229
<211> 28
<212> PRT
<213> Homo sapiens

<400> 229

Leu Trp His Gly Ser Arg Thr Thr Asn Phe Ala Gly Ile Leu Ser Gln
1 5 10 15

Gly Leu Arg Ile Ala Pro Pro Glu Ala Pro Val Thr
20 25

<210> 230
<211> 16
<212> PRT
<213> Homo sapiens

<400> 230

Met Ala Val Thr Ala Leu Ala Ala Arg Thr Trp Leu Gly Val Trp Gly
1 5 10 15

<210> 231
<211> 28
<212> PRT
<213> Homo sapiens

<400> 231

Arg Asp Glu Glu Ser Thr Arg Ser Glu Glu Val Thr Arg Glu Glu Met
1 5 10 15

Ala Ala Ala Gly Leu Thr Val Thr Val Thr His Ser
20 25

<210> 232
<211> 19
<212> PRT
<213> Homo sapiens

<400> 232

Glu Val Thr Arg Glu Glu Met Ala Ala Ala Gly Leu Thr Val Thr Val
1 5 10 15

Thr His Ser

<210> 233
<211> 22

<212> PRT
<213> Homo sapiens

<400> 233

Tyr Ala Lys Ser Gln Pro Asp Met Ala Ile Met Ala Val Asn Thr Phe
1 5 10 15

Val Lys Asp Cys Glu Asp
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<210> 234
<211> 16
<212> PRT
<213> Homo sapiens

<400> 234

Pro Asp Met Ala Ile Met Ala Val Asn Thr Phe Val Lys Asp Cys Glu
1 5 10 15

<210> 235
<211> 14
<212> PRT
<213> Homo sapiens

<400> 235

Ala Pro Ala Thr Ser Glu Gly Gln Ser Lys Arg Cys Lys Pro
1 5 10

<210> 236
<211> 16
<212> PRT
<213> Homo sapiens

<400> 236

Glu Ile Ser Leu Leu Lys Glu Leu Asn His Pro Asn Ile Val Lys Leu
1 5 10 15

<210> 237
<211> 19
<212> PRT
<213> Homo sapiens

<400> 237

Tyr Thr His Glu Val Val Thr Leu Trp Tyr Arg Ala Pro Glu Ile Leu
1 5 10 15

Leu Gly Cys

<210> 238
<211> 17
<212> PRT

<213> Homo sapiens

<400> 238

Pro Glu Leu Val His Tyr Arg Glu Glu Lys His Val Phe Pro Gln Arg
1 5 10 15

Phe

<210> 239
<211> 11
<212> PRT
<213> Homo sapiens

<400> 239

Lys Met Ala Ala Ala Lys Cys Arg Asn Arg Arg
1 5 10

<210> 240
<211> 23
<212> PRT
<213> Homo sapiens

<400> 240

Val Gln Ala Gln Pro Gln Ile Ala Thr Leu Ala Gln Val Ser Met Pro
1 5 10 15

Ala Ala His Ala Thr Ser Ser
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<210> 241
<211> 36
<212> PRT
<213> Homo sapiens

<400> 241

Met Arg Lys Ile Val Ala Thr Trp Met Leu Glu Val Cys Glu Glu Gln
1 5 10 15

Lys Cys Glu Glu Glu Val Phe Pro Leu Ala Met Asn Tyr Leu Asp Arg
20 25 30

Phe Leu Ser Leu
35

<210> 242
<211> 16
<212> PRT
<213> Homo sapiens

<400> 242

Ala Lys Ser Lys Gln Ala Ile Leu Ala Ala Gln Arg Arg Gly Gly Asp

1 5 10 15

<210> 243
<211> 14
<212> PRT
<213> Homo sapiens

<400> 243

Arg Gly Gly Ala Asp Asp Glu Arg Ser Ser Trp Arg Asn Ala
1 5 10

<210> 244
<211> 27
<212> PRT
<213> Homo sapiens

<400> 244

His Tyr Glu Asp His Ser Val Ala Asp Ala Ala Met Glu Gln Tyr Ile
1 5 10 15

Leu Tyr Leu Val Glu His Glu Glu Tyr Gln Leu
20 25

<210> 245
<211> 12
<212> PRT
<213> Homo sapiens

<400> 245

Pro Gln Leu Lys Ser Gly Glu Leu Ala Lys Arg Ser
1 5 10

<210> 246
<211> 44
<212> PRT
<213> Homo sapiens

<400> 246

Val Thr Val Ser Ala Asp Ser Ser Ala Ser Met Asn Ser Gly Val Leu
1 5 10 15

Leu Val Arg Pro Ser Arg Leu Ser Ser Ser Gly Thr Pro Met Leu Ala
20 25 30

Gly Val Ser Glu Tyr Glu Leu Pro Glu Asp Pro Arg
35 40

<210> 247
<211> 20
<212> PRT
<213> Homo sapiens

<400> 247

Gly Thr Lys Lys Ser Asp Phe His Ser Gln Met Ala Val His Lys Leu
1 5 10 15

Ala Lys Ser Ile
20

<210> 248
<211> 19
<212> PRT
<213> Homo sapiens

<400> 248

Leu Thr Ser Gln Thr Met Gly Gly Gln Ala Glu Thr Leu Leu Thr Ser
1 5 10 15

Gln Lys Gly

<210> 249
<211> 16
<212> PRT
<213> Homo sapiens

<400> 249

Ile Lys Pro Ile Ser Ile Ala Gly Gly Phe Tyr Gly Glu Glu Pro Leu
1 5 10 15

<210> 250
<211> 20
<212> PRT
<213> Homo sapiens

<400> 250

Asp Gln Gln Glu Ala Ala Leu Val Asp Met Val Asn Asp Gly Val Glu
1 5 10 15

Asp Leu Arg Cys
20

<210> 251
<211> 41
<212> PRT
<213> Homo sapiens

<400> 251

Cys Leu Asp Ala Phe Pro Leu Leu Ser Ala Tyr Val Gly Arg Leu Ser
1 5 10 15

Ala Arg Pro Lys Leu Lys Ala Phe Leu Ala Ser Pro Glu Tyr Val Asn
20 25 30

Leu Pro Ile Asn Gly Asn Gly Lys Gln

35 40

<210> 252
<211> 39
<212> PRT
<213> Homo sapiens

<400> 252

Trp Met Asp Gly Arg Asp Glu Val Thr Gln Gln Lys Tyr Gln Arg Pro
1 5 10 15

Glu Thr Glu Trp Pro Arg Val Ser Leu His Pro Glu Pro Glu Asp Ala
20 25 30

Ala Lys Thr Ser Leu Ser Glu
35

<210> 253
<211> 25
<212> PRT
<213> Homo sapiens

<400> 253

Arg Ala Ala Pro Gly Asn Gln Pro Ser Gln Ala Pro Ala Pro Phe Leu
1 5 10 15

Lys Lys Leu Leu Gly Thr Leu Gln Leu
20 25

<210> 254
<211> 27
<212> PRT
<213> Homo sapiens

<400> 254

Ile Ser Asp Gln Asp Gln Glu Val Thr Leu Glu Glu Asp Leu Met Asp
1 5 10 15

Met Ala Val Asp Val Asp Leu Gly Met Ala Ile
20 25

<210> 255
<211> 53
<212> PRT
<213> Homo sapiens

<400> 255

Ala Gly Leu Glu Arg Met Arg Pro Gly Ala Tyr Ser Thr Gly Tyr Gly
1 5 10 15

Gly Tyr Glu Glu Tyr Ser Gly Leu Ser Asp Gly Tyr Gly Phe Thr Thr
20 25 30

Asp Leu Phe Gly Arg Asp Leu Ser Tyr Cys Leu Ser Gly Met Tyr Asp
35 40 45

His Arg Tyr Gly Asp
50

<210> 256
<211> 16
<212> PRT
<213> Homo sapiens

<400> 256

Gly Val Gly Ala Gly Glu Asp Gly Gly Ser Arg Gly Arg Glu Leu His
1 5 10 15

<210> 257
<211> 17
<212> PRT
<213> Homo sapiens

<400> 257

Lys Asp Gln Val Ala Asn Ser Ala Phe Val Glu Arg Leu Arg Lys His
1 5 10 15

Gly

<210> 258
<211> 19
<212> PRT
<213> Homo sapiens

<400> 258

Met Ser Ser Phe Gly Tyr Arg Thr Leu Thr Val Ala Leu Phe Thr Leu
1 5 10 15

Ile Cys Cys

<210> 259
<211> 14
<212> PRT
<213> Homo sapiens

<400> 259

Tyr Glu Pro Val Ser Asp Ser Gln Met Val Ile Ile Val Thr
1 5 10

<210> 260
<211> 13
<212> PRT
<213> Homo sapiens

<400> 260

Lys Leu Val Arg Lys Asp Arg Leu Val Leu Ser Tyr Val
1 5 10

<210> 261
<211> 16
<212> PRT
<213> Homo sapiens

<400> 261

Lys Lys Arg Tyr Asp Leu Ser Ala Leu Val Arg His Ala Glu Pro Glu
1 5 10 15

<210> 262
<211> 45
<212> PRT
<213> Homo sapiens

<400> 262

Leu Leu Gly Leu Cys Ala Leu Leu Pro Arg Leu Ala Gly Leu Asn Ile
1 5 10 15

Cys Thr Ser Gly Ser Ala Thr Ser Cys Glu Glu Cys Leu Leu Ile His
20 25 30

Pro Lys Cys Ala Trp Cys Ser Lys Glu Asp Phe Gly Ser
35 40 45

<210> 263
<211> 37
<212> PRT
<213> Homo sapiens

<400> 263

Lys Asp Cys Val Met Met Phe Thr Tyr Val Glu Leu Pro Ser Gly Lys
1 5 10 15

Ser Asn Leu Thr Val Leu Arg Glu Pro Glu Cys Gly Asn Thr Pro Asn
20 25 30

Ala Met Thr Ile Leu
35

<210> 264
<211> 29
<212> PRT
<213> Homo sapiens

<400> 264

Gly His Gly Glu Cys His Cys Gly Glu Cys Lys Cys His Ala Gly Tyr
1 5 10 15

Ile Gly Asp Asn Cys Asn Cys Ser Thr Asp Ile Ser Thr
20 25

<210> 265
<211> 30
<212> PRT
<213> Homo sapiens

<400> 265

Val Glu Leu Pro Ser Gly Lys Ser Asn Leu Thr Val Leu Arg Glu Pro
1 5 10 15

Glu Cys Gly Asn Thr Pro Asn Ala Met Thr Ile Leu Leu Ala
20 25 30

<210> 266
<211> 24
<212> PRT
<213> Homo sapiens

<400> 266

Pro Glu Ala Ala Val Arg Glu Thr Val Met Leu Leu Leu Cys Leu Gly
1 5 10 15

Val Pro Thr Gly Arg Pro Tyr Asn
20

<210> 267
<211> 16
<212> PRT
<213> Homo sapiens

<400> 267

Gly Tyr Gly Arg Ala Pro Gly Gly Leu Ser Leu His Asp Tyr Lys Leu
1 5 10 15

<210> 268
<211> 9
<212> PRT
<213> Homo sapiens

<400> 268

Pro Gly Gly Leu Ser Leu His Asp Tyr
1 5

<210> 269
<211> 35
<212> PRT
<213> Homo sapiens

<400> 269

Ser Val Gln Trp Leu Asp Glu Asp Gly Thr Thr Val Leu Gln Asp Glu

1 5 10 15

Arg Phe Phe Pro Tyr Ala Asn Gly Thr Leu Gly Ile Arg Asp Leu Gln
20 25 30

Ala Asn Asp
35

<210> 270
<211> 36
<212> PRT
<213> Homo sapiens

<400> 270

Thr Arg Thr Ile Ile Gln Lys Glu Pro Ile Asp Leu Arg Val Lys Ala
1 5 10 15

Thr Asn Ser Met Ile Asp Arg Lys Pro Arg Leu Leu Phe Pro Thr Asn
20 25 30

Ser Ser Ser His
35

<210> 271
<211> 30
<212> PRT
<213> Homo sapiens

<400> 271

Gly Asn Leu Tyr Phe Ala Asn Val Leu Thr Ser Asp Asn His Ser Asp
1 5 10 15

Tyr Ile Cys His Ala His Phe Pro Gly Thr Arg Thr Ile Ile
20 25 30

<210> 272
<211> 20
<212> PRT
<213> Homo sapiens

<400> 272

Ala Phe Gly Ala Pro Val Pro Ser Val Gln Trp Leu Asp Glu Asp Gly
1 5 10 15

Thr Thr Val Leu
20

<210> 273
<211> 15
<212> PRT
<213> Homo sapiens

<400> 273

Glu Tyr Glu Gly His His Val Met Glu Pro Pro Val Ile Thr Glu
1 5 10 15

<210> 274
<211> 13
<212> PRT
<213> Homo sapiens

<400> 274

Lys Glu Glu Leu Gly Val Thr Val Tyr Gln Ser Pro His
1 5 10

<210> 275
<211> 17
<212> PRT
<213> Homo sapiens

<400> 275

Asp Arg Lys Pro Arg Leu Leu Phe Pro Thr Asn Ser Ser Ser His Leu
1 5 10 15

Val

<210> 276
<211> 19
<212> PRT
<213> Homo sapiens

<400> 276

Glu Ala Lys Asn Leu Val Ser Glu Ala Ile Ala Ala Gly Ile Phe Asn
1 5 10 15

Asp Leu Gly

<210> 277
<211> 25
<212> PRT
<213> Homo sapiens

<400> 277

Pro Leu Gly Ser Ala Val Asp Ile Leu Ala Thr Asp Asp Pro Asn Phe
1 5 10 15

Ser Gln Glu Asp Gln Gln Asp Thr Gln
20 25

<210> 278
<211> 14
<212> PRT
<213> Homo sapiens

<400> 278

Leu Ser Lys Met Gln Asp Asp Asn Gln Val Met Val Ser Glu
1 5 10

<210> 279
<211> 22
<212> PRT
<213> Homo sapiens

<400> 279

Pro Ala Pro Val Asn Gly Ile Asn Gly Tyr Asn Glu Asp Ile Asn Gln
1 5 10 15

Glu Ser Ala Pro Lys Ala
20

<210> 280
<211> 65
<212> PRT
<213> Homo sapiens

<400> 280

Tyr Ser Val His Asn Lys Thr Gly Ala Lys Leu Pro Val Lys Trp Met
1 5 10 15

Ala Leu Glu Ser Leu Gln Thr Gln Lys Phe Thr Thr Lys Ser Asp Val
20 25 30

Trp Ser Phe Gly Val Val Leu Trp Glu Leu Met Thr Arg Gly Ala Pro
35 40 45

Pro Tyr Pro Asp Val Asn Thr Phe Asp Ile Thr Val Tyr Leu Leu Gln
50 55 60

Gly
65

<210> 281
<211> 22
<212> PRT
<213> Homo sapiens

<400> 281

Met Cys Asp Phe Thr Glu Asp Gln Thr Thr Glu Phe Lys Glu Ala Phe
1 5 10 15

Gln Leu Phe Asp Arg Thr
20

<210> 282
<211> 26
<212> PRT
<213> Homo sapiens

<400> 282

Glu Asp Gln Thr Thr Glu Phe Lys Glu Ala Phe Gln Leu Phe Asp Arg
1 5 10 15

Thr Gly Asp Gly Lys Ile Leu Tyr Asn Gln
20 25

<210> 283
<211> 24
<212> PRT
<213> Homo sapiens

<400> 283

Ser Thr Ser Val Pro Glu Asn Pro Lys Ser Ser Ala Ser Thr Ala Val
1 5 10 15

Ser Ala Ala Pro Thr Glu Lys Glu
20

<210> 284
<211> 19
<212> PRT
<213> Homo sapiens

<400> 284

Glu Leu Thr Ser Gln Ala Glu Arg Ala Glu Glu Leu Gly Gln Glu Leu
1 5 10 15

Lys Ala Trp

<210> 285
<211> 23
<212> PRT
<213> Homo sapiens

<400> 285

Asn Leu Lys Arg Tyr Leu Lys Ser Glu Pro Ile Pro Glu Ser Asn Asp
1 5 10 15

Gly Pro Val Lys Val Val Val
20

<210> 286
<211> 18
<212> PRT
<213> Homo sapiens

<400> 286

Ala Pro Glu Lys Pro Val Lys Lys Gln Lys Thr Gly Glu Thr Ser Arg
1 5 10 15

Ala Leu

<210> 287
<211> 42
<212> PRT
<213> Homo sapiens

<400> 287

Gly Ile Asn Gln Lys Leu Leu Ala Glu Ala Leu Asn Gln Val Thr Gln
1 5 10 15

Arg Ala Ser Arg Arg Ser Asp Ser Ala Ser Ser Glu Pro Val Gly Ile
20 25 30

Tyr Gln Gly Phe Glu Lys Lys Thr Gly Val
35 40

<210> 288
<211> 36
<212> PRT
<213> Homo sapiens

<400> 288

Ile Ile Gly Arg Cys Thr Gly Thr Ala Ala Asn Ser Arg Asp Thr Ile
1 5 10 15

Phe Gln Lys Glu Arg Phe Asn Ile Asp Met Pro His Arg Phe Lys Val
20 25 30

His Asn Tyr Met
35

<210> 289
<211> 30
<212> PRT
<213> Homo sapiens

<400> 289

Gly Gln Thr Ser Thr Lys Gln Lys Thr Asn Lys Pro Thr Tyr Asn Glu
1 5 10 15

Glu Phe Cys Ala Asn Val Thr Asp Gly Gly His Leu Glu Leu
20 25 30

<210> 290
<211> 35
<212> PRT
<213> Homo sapiens

<400> 290

Cys Ala Asn Val Thr Asp Gly Gly His Leu Glu Leu Ala Val Phe His

1 5 10 15

Glu Thr Pro Leu Gly Tyr Asp His Phe Val Ala Asn Cys Thr Leu Gln
20 25 30

Phe Gln Glu
35

<210> 291
<211> 19
<212> PRT
<213> Homo sapiens

<400> 291

Gly His Leu Phe Gln Ala Lys Arg Phe Asn Arg Arg Ala Tyr Cys Gly
1 5 10 15

Gln Cys Ser

<210> 292
<211> 40
<212> PRT
<213> Homo sapiens

<400> 292

Pro Leu Thr Leu Lys Trp Val Asp Ser Glu Gly Asp Pro Cys Thr Val
1 5 10 15

Ser Ser Gln Met Glu Leu Glu Glu Ala Phe Arg Leu Ala Arg Gln Cys
20 25 30

Arg Asp Glu Gly Leu Ile Ile His
35 40

<210> 293
<211> 31
<212> PRT
<213> Homo sapiens

<400> 293

Val Thr Gln Phe Asn Lys Val Ala Gly Ala Val Val Ser Ser Val Leu
1 5 10 15

Gly Ala Thr Ser Thr Gly Glu Gly Pro Gly Glu Val Thr Ile Arg
20 25 30

<210> 294
<211> 36
<212> PRT
<213> Homo sapiens

<400> 294

Asn Leu Glu Asn Pro Ser Cys Asn Ser Val Leu Asp Pro Arg Asp Leu
1 5 10 15

Arg Gln Leu His Gln Met Ser Ser Ser Ser Met Ser Ala Gly Ala Asn
20 25 30

Ser Asn Thr Thr
35

<210> 295
<211> 47
<212> PRT
<213> Homo sapiens

<400> 295

Glu Gln Ser Gln Ala Pro Ile Thr Pro Gln Gln Gly Gln Ala Leu Ala
1 5 10 15

Lys Gln Ile His Ala Val Arg Tyr Leu Glu Cys Ser Ala Leu Gln Gln
20 25 30

Asp Gly Val Lys Glu Val Phe Ala Glu Ala Val Arg Ala Val Leu
35 40 45

<210> 296
<211> 20
<212> PRT
<213> Homo sapiens

<400> 296

Gly Trp Met Glu Glu Gln Ser Gln Ala Pro Ile Thr Pro Gln Gln Gly
1 5 10 15

Gln Ala Leu Glu
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<210> 297
<211> 35
<212> PRT
<213> Homo sapiens

<400> 297

Lys Glu Gln Ser Gln Ala Pro Ile Thr Pro Gln Gln Gly Gln Ala Leu
1 5 10 15

Ala Lys Gln Ile His Ala Val Arg Tyr Leu Glu Cys Ser Ala Leu Gln
20 25 30

Gln Asp Gly
35

<210> 298
<211> 18
<212> PRT

<213> Homo sapiens

<400> 298

Thr Pro Gln Gln Gly Gln Ala Leu Ala Lys Gln Ile His Ala Val Arg
1 5 10 15

Tyr Leu

<210> 299
<211> 20
<212> PRT
<213> Homo sapiens

<400> 299

Ser Arg Ile Arg Val His Leu Thr Pro Ala Ala Ser Thr Met Leu Pro
1 5 10 15

Lys Phe Asn Pro
20

<210> 300
<211> 17
<212> PRT
<213> Homo sapiens

<400> 300

Gly Glu Lys Lys Lys Gly Arg Ser Ala Ile Asn Glu Val Val Thr Arg
1 5 10 15

Glu

<210> 301
<211> 22
<212> PRT
<213> Homo sapiens

<400> 301

Trp Met Asp Gly Arg Met Lys Lys Val Arg Gly Thr Ala Lys Ala Asn
1 5 10 15

Val Gly Ala Gly Lys Lys
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<210> 302
<211> 40
<212> PRT
<213> Homo sapiens

<400> 302

Glu Ser Gln Glu His Pro Glu Ala Asp Pro Gly Ser Ala Ala Pro Tyr

1 5 10 15

Leu Lys Thr Lys Phe Ile Cys Val Thr Pro Thr Thr Cys Ser Asn Thr
20 25 30

Ile Asp Leu Pro Met Ser Pro Arg
35 40

<210> 303
<211> 32
<212> PRT
<213> Homo sapiens

<400> 303

Asp Val Arg Lys Arg Val Gln Asp Leu Glu Gln Lys Met Lys Val Val
1 5 10 15

Glu Asn Leu Gln Asp Asp Phe Asp Phe Asn Tyr Lys Thr Leu Lys Ser
20 25 30

<210> 304
<211> 38
<212> PRT
<213> Homo sapiens

<400> 304

Phe Leu Gln Glu Ser Asn Val Leu Tyr Gln His Asn Leu Arg Arg Ile
1 5 10 15

Lys Gln Phe Leu Gln Ser Arg Tyr Leu Glu Lys Pro Met Glu Ile Ala
20 25 30

Arg Ile Val Ala Arg Cys
35

<210> 305
<211> 15
<212> PRT
<213> Homo sapiens

<400> 305

Asp Gln Gln Tyr Ser Arg Phe Leu Gln Glu Ser Asn Val Leu Tyr
1 5 10 15

<210> 306
<211> 35
<212> PRT
<213> Homo sapiens

<400> 306

Asn Lys Gln Gln Ala His Asp Leu Leu Ile Asn Lys Pro Asp Gly Thr
1 5 10 15

Phe Leu Leu Arg Phe Ser Asp Ser Glu Ile Gly Gly Ile Thr Ile Ala
20 25 30

Trp Lys Phe
35

<210> 307
<211> 34
<212> PRT
<213> Homo sapiens

<400> 307

Lys Arg Ile Lys Arg Ser Asp Arg Arg Gly Ala Glu Ser Val Thr Glu
1 5 10 15

Glu Lys Phe Thr Ile Leu Phe Glu Ser Gln Phe Ser Val Gly Gly Asn
20 25 30

Glu Leu

<210> 308
<211> 15
<212> PRT
<213> Homo sapiens

<400> 308

Thr Ile Leu Phe Glu Ser Gln Phe Ser Val Gly Gly Asn Glu Leu
1 5 10 15

<210> 309
<211> 40
<212> PRT
<213> Homo sapiens

<400> 309

Gln His Gly Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln
1 5 10 15

Ser Cys Glu Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu Trp
20 25 30

Arg Tyr Asn Ser Cys Ala Pro Ala
35 40

<210> 310
<211> 28
<212> PRT
<213> Homo sapiens

<400> 310

Ala Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp
1 5 10 15

His Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met
20 25

<210> 311
<211> 24
<212> PRT
<213> Homo sapiens

<400> 311

Cys Cys Gly Arg Cys Leu Pro Ser Ala Cys Glu Val Val Thr Gly Ser
1 5 10 15

Pro Arg Gly Asp Ser Gln Ser Ser
20

<210> 312
<211> 20
<212> PRT
<213> Homo sapiens

<400> 312

Val Ser Gln Gly Asp Arg Glu Gln Ala Pro Asn Leu Val Tyr Met Val
1 5 10 15

Thr Gly Asn Pro
20

<210> 313
<211> 21
<212> PRT
<213> Homo sapiens

<400> 313

Cys His Thr Val Thr Cys Gln Pro Asp Gly Gln Thr Leu Leu Lys Ser
1 5 10 15

His Arg Val Asn Cys
20

<210> 314
<211> 21
<212> PRT
<213> Homo sapiens

<400> 314

Ser Thr Ser Glu Val Leu Lys Tyr Thr Leu Phe Gln Ile Phe Ser Lys
1 5 10 15

Ile Asp Arg Pro Glu
20